METHOD OF DETECTING AND TREATING TUBEROUS SCLEROSIS COMPLEX ASSOCIATED DISORDERS

RELATED APPLICATIONS

This application claims priority from USSN 60/254,268, filed December 8, 2000, which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates to methods of detecting and treating Tuberous Sclerosis Complex (TSC) associated disorders.

BACKGROUND OF THE INVENTION

The phakomatoses, or 'neuro-cutaneous disorders', are a group of three Mendelian autosomal dominantly inherited diseases that present with phenotypes affecting multiple organ systems in affected individuals. Neuro-cutaneous disorders include for example, Neurofibromatosis (NF), Tuberous Sclerosis (TSC) and Von Hippel-Lindau (VHL). These diseases all produce both neurological and dermatological symptoms.

Tuberous sclerosis complex (TSC) is an autosomal dominant tumor-suppressor gene syndrome, characterized by development of distinctive benign tumors (hamartomas) and malformations (hamartias) in multiple organ systems. The brain, skin, heart, and kidneys are commonly affected. TSC lesions occurring in the skin and kidney contain smooth muscle cells, endothelial cells, adipocytes, and large neuronal appearing cells. Despite this complex cellular architecture, kidney and other lesions in TSC appear to be clonal in nature, based on clonality and loss of heterozygosity (LOH) analyses. In the brain, TSC produces both subependymal tubers that line the ventricular sacs and subcortical hamartomas which serve as foci for epileptic discharges. TSC produces cardiac rhabdomyomas in the fetus/newborn that spontaneously regress in the first year of life. TSC is also associated with renal angiomyolipomas, pulmonary symptoms, and manifestations in other organ systems. In addition, TSC is also associated with multiple dermatological features such as hypomelanotic macules, facial angiofibroma, shagreen patches, and ungual fibromas.

A better understanding of the molecular nature of this disease will provide new therapeutic

tools to treat the pathologies associated with TSC complex not only in TSC patients but also in non TSC patients afflicted by similar pathologies.

SUMMARY OF THE INVENTION

The present invention is based in part on the discovery of changes in expression patterns of multiple nucleic acid sequences in cells derived from the Tsc2 knockout transgenic mice compared to the expression pattern found in cells derived from Tsc2+/– heterozygote and wild type sibling mice. These differentially expressed nucleic acids include previously undescribed sequences and nucleic acids sequences that, while previously described, have not heretofore been identified as TSC modulated.

In various aspects, the invention includes methods of diagnosing or determining susceptibility to Tuberous Sclerosis Complex (TSC) associated disorder, and methods of treating those disorders. For example, in one aspect, the invention provides a method of diagnosing determining susceptibility to a tuberous sclerosis complex associated disorder by providing a test cell population that includes one or more cells capable of expressing one or more TSC modulated nucleic acids sequences. Levels of expression of one or more sequences, termed TSCX sequences, are then compared to the levels of expression of the corresponding nucleic acids in a reference cell population. The reference cell population contains cells whose tuberous sclerosis complex associated disorder status is known, *i.e.,* the reference cells are known to have or are known not to have a tuberous sclerosis associated disorder.

The invention in another aspect includes a method of identifying a therapeutic agent for treating a tuberous sclerosis complex associated disorder. The method includes providing from the subject a test cell population comprising a cell capable of expressing one or more TSCX nucleic acids sequences, contacting the test cell population with the therapeutic agent, and comparing the expression of the nucleic acids sequences in the test cell population to the expression of the nucleic acids sequences in a reference cell population.

The invention in a further aspect includes a method of selecting an individualized therapeutic agent appropriate for a particular subject. The method includes providing from the subject a test cell population comprising a cell capable of expressing one or more TSCX nucleic acids sequences, contacting the test cell population with the therapeutic agent, and comparing the

expression of the nucleic acids sequences in the test cell population to the expression of the nucleic acids sequences in a reference cell population.

Also provided are novel nucleic acids, as well as their encoded polypeptides, which are tuberous sclerosis complex modulated.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Other features and advantages of the invention will be apparent from the following detailed description and claims.

DETAILED DESCRIPTION

The present invention is based in part on the discovery of changes in expression patterns of multiple nucleic acid sequences in cells derived from the Tsc2 knockout transgenic mice compared to the expression pattern found in cells derived from Tsc2+/– heterozygote and wild type sibling mice.

The change is expression pattern was identified by GeneCalling™ analysis (U. S. Patent No. 5,871,697 ; Shimkets et al., 1999 Nature Biotechnology 17:198-803, incorporated herein by reference in their entireties) of neuronal stem cell (NSC) and mouse emroyonic fibroblasts (MEF) cell lines established from 10-11 day embryos from mice of the three genotypes (i.e.,)

A summary of the sequences analyzed are presented in Table 1. The 142 single nucleic acid sequences identified herein, are referred to herein as TSC 1-142 or TSCX nucleic acids or polypeptided. Differential expression of TSC 1-142 gene fragments was confirmed using a unlabeled oligonucleotide competition assay as described in Shimkets et al., Nature Biotechnology 17:198-803.

By comparing the genes differentially expressed in both cell lines it was possible to

identify understand common mechanisms in TSC -/- tumor formation. Whereas, by comparing the the genes differentially expressed in NSC cell lines it was identify genes that are expressed in cells that are the originators (*i.e.,* progentitors) of TSC tumors. Based on the TSC phenotype, genes that are up-regulated in the TSC- cells may have a role in cancer progression, specifically for renal and lung carcinomas

Twenty-six sequences (TSC: 1-26) represent novel murine genes for which the sequence identity to sequences found in public databases suggesting a putative homology.

The 116other sequenced identified have been previously described. For some of the novel sequences (*i.e.,* TSC: 1-26), a cloned sequence is provided along with one or more additional sequence fragments (*e.g.*, ESTs or contigs) which contain sequences substantially identical to, the cloned sequence. Also provided is a consensus sequences which includes a composite sequence assembled from the cloned and additional fragments. For a given TSC sequence, its expression can be measured using any of the associated nucleic acid sequences may be used in the methods described herein. For previously described sequences database accession numbers are provided. This information allows for one of ordinary skill in the art to deduce information necessary for detecting and measuring expression of the TSC nucleic acid sequences.

A subset of the TSC modulated genes can be further subdivided into three classes:

A. <u>Secreted and/or membrane bound proteins that are up-regulated in cell derived from Tsc2 knockout transgenic mice</u>

Proteins in this catagory include, Plasma phospholipid transfer protein, Lysyl hydroxylase isoform 2, DVS27-related protein [AB024518], Cathepsin L, Tenascin, ADAMTS1, Tissue inhibitor of metalloproteinase-2, Integrin beta-5, Thrombospondin 2 (THBS2) Aspartyl protease 1, Cyr61, Tetraspan NET-7, Cysteine-rich glycoprotein SPARC, neuronal pentraxin receptor, ITM2B - E25B protein Integral Membrane Protein 2B, transmembrane glycoprotein NMB, and zinc finger protein

These proteins are potential candidates for antibody screening and antibody-binding therapy for the treatment of TSC and TSC related diseases.

B. <u>Secreted and/or membrane bound proteins that are down-regulated in cell derived from Tsc2 knockout transgenic mice</u>

Proteins in this catacory include, Growth/differentiation factor 1 (GDF-1), Extracellular matrix associated protein (Sc1), Membrane-type 2 matrix metalloproteinase and Thrombospondin 1 mice.

These proteins that are potentail canidates for the treatment of TSC and TSC related diseases.

C. Protein with enzymatic activies

Proteins in this catory include Growth factor-inducible immediate early gene 3CH134 /erp, Galactokinase 1, Serum inducible kinase (SNK), PAF acetylhydrolase Aspartyl protease 1, Lysyl hydroxylase isoform 2 Peroxisomal D2, and D4-dienoyl-CoA reductase (Pdcr) .

These proteins are potential candidates for small molecule screening and small molecule drug therapy for the treatment of TSC and TSC related diseases.

The TSC modulated nucleic acids discussed herein include the following:

Table 1

Gene Discovered	TSCX Assignment	SEQ ID NO	Acc #	MEF +/- TSC2 vs. +/+ TSC2 (16606)	MEF -/- TSC2 vs. +/+ TSC2 (16607)	NSC +/- TSC2 vs. +/+ TSC2 (16608)	NSC -/- TSC2 vs. +/+ TSC2 (16609)
Novel gene fragment, 2520 bp	1	1	aa914498	±1.0	+1.5	+2	+1.5
Novel gene fragment, 1863 bp	2	2	aa073509	±1.0	-6	-2	-2
Novel gene fragment, 750 bp	3	3	AA183535	±1.0	+3	±1.0	+4
Novel gene fragment, 281 bp, 91% AA identity to rat Steroid sensitivity gene-1 protein [AAF35351]	4	4		±1.0	-1.5	±1.0	NEW
Novel gene fragment, 1568 bp, 86% SI to human Tetraspan NET-7 [AF120266]/ old brain study also	5	5		±1.0	X	+2	+6
Novel gene fragment, 300 bp, 94% SI to rat 10-formyltetrahydrofolate dehydrogenase [M59861]	6	6		O	O	O	+15
Novel gene fragment, 965 bp, 86% SI to rat myr3 myosin I heavy chain [X74815]	7	7		-2	X	±1.0	NEW
Novel gene fragment, 408 bp, 97% SI to rat Limbic system-associated membrane protein [U31554]	8	8		O	O	±1.0	OFF
Novel gene fragment, 777 bp, 83% SI to rat neuronal pentraxin receptor [AF005099]	9			±1.0	±1.0	±1.0	NEW
Novel gene fragment, 354 bp, 87% SI to human KIAA0631 [AB014531]	10	9		±1.0	X	-2	-5
Novel gene fragment, 955 bp	11	10		±1.0	X	-3	-8
Novel gene fragment, 1113 bp	12	11		+2	X	±1.0	-9

Novel gene fragment, 918 bp	13			±1.0	**±1.0**	±1.0	**+3**
Novel gene fragment, 1166 bp	14			±1.0	±1.0	±1.0	**+10**
Novel gene fragment, 594 bp	15	12		±1.0	±1.0	±1.0	**-10**
Novel gene fragment, 713 bp	16	13		O	O	±1.0	**OFF**
Novel gene fragment, 306 bp, 95% SI to rat ribosomal protein L13a [X68282]	17	14		±1.0	**-2**	±1.0	**X**
Novel gene fragment, 66 bp, 96% SI to rat ribosomal protein S20 [X51537]	18	15		±1.0	**-2**	-2	**±1.0**
Novel gene fragment, 1613 bp	19	16		±1.0	**+3**	±1.0	**-5**
Novel gene fragment, 2245 bp	20	17		±1.0	**NEW**	-2	**-3**
Novel gene fragment, 171 bp, 86% SI to rat nonmuscle caldesmon [U18419]	21	18			**±1.0**		**+1.5**
Novel gene fragment, 491 bp, 72 % SI to human DVS27-related protein [AB024518]	22	19					**+10**
Novel gene fragment, 659 bp, 72% SI to human ATP cassette binding transporter 1 [AF165281]	23	20		-2	**X**	±1.0	**NEW**
Novel gene fragment, 341 bp, 84% SI to human sorting nexin 5 (SNX5) [AF121855]	24	21					
Novel gene fragment, 53 bp, 84% SI to rat calcium-independent alpha-latrotoxin receptor [U72487]	25	22					
Novel gene fragment, 52 bp, 98% SI to rat Na+,K+-ATPase alpha(+) isoform catalytic subunit [M14512]	26			-2			
MEF & NSC -/- conserved differential expression							
Ribosomal protein L8 (RPL8)	27		U67771	-9	**OFF**	-3	**OFF**
Alpha-B crystallin (p23)	28		M63170	±1.0	**+20**	+2	**+7**
Tumor cell dnaJ-like protein 1	29		L16953	±1.0	**+2**	+3	**+2**
Insulin-like growth factor-binding protein-4	30		S80566	±1.0	**-2**	+3	**OFF**
Insulin-like growth factor binding protein 5 (IGFBP5)	31		L12447	±1.0	**NEW**	+2	**+5**
Rac1	32		X57277	-2	**-1.5**	-2	**-2**
Growth factor-inducible immediate early gene 3CH134 /erp	33		S64851	±1.0	**+2**	±1.0	**+6**
Phosphatidic acid phosphatase type 2c (Ppap2c)	34		AF123611	±1.0	**-5**	±1.0	**-4**
Annexin III	35		AJ001633	±1.0	**NEW**	±1.0	**NEW**
Taipoxin-associated calcium binding protein 49	36		AF049125	±1.0	**-2**	±1.0	**OFF**
C-fos oncogene	37		V00727	+2	**+1.5**	±1.0	**NEW**
Stra13	38		AF010305	+2	**+6**	±1.0	**+2**

E1B 19K/Bcl-2-binding protein homolog (Nip3)	39		AF041054	+2	+5	±1.0	+3
Peroxisomal D2,D4-dienoyl-CoA reductase (Pdcr)	40		AF155575	+7	NEW	+2	NEW
Galactokinase 1	41		AB027012	±1.0	+4	±1.0	+1.5
Alpha-enolase (2-phospho-D-glycerate hydrolase) (NNE)	42		X52379	+3	+5	+3	+15
Alpha-N-acetylglucosaminidase	43		AF003255	±1.0	+2	±1.0	+3
Uncoupling protein 2 (UCP2)	44		AF111998	±1.0	NEW	+2	NEW
ANC1 for adenine nucleotide carrier	45		X74510	±1.0	-1.5	±1.0	-2
Vacuolar ATPase subunit A gene	46		U13837	±1.0	+3	±1.0	+2
S-adenosylmethionine decarboxylase	47		D12780	±1.0	+2	±1.0	+5
Spermidine/spermine N1-acetyltransferase (SSAT)	48		L10244	±1.0	+5	±1.0	+4
Xanthine dehydrogenase	49		X62932	±1.0	+9	±1.0	NEW
MBOCT	50		AB012808	±1.0	OFF	±1.0	-3
Plasma phospholipid transfer protein	51		U37226	±1.0	+2	-2	+5
Lysyl hydroxylase isoform 2	52		AF080572	+3	+6	±1.0	NEW
Cathepsin L	53		J02583	±1.0	+5	±1.0	+4
Ezrin	54		X60671	+2	+4	±1.0	+4
Thy-1.2 glycoprotein	55		M12379	-2	-4	±1.0	-10
A-X actin	56		J04181	+5	NEW	+6	NEW
MHC class I heavy chain precursor (H-2D(b))	57		U47325	+3	+2	±1.0	+4
MHC class I heavy chain precursor (H-2K(b))	58		U47328	+2	NEW	±1.0	+3
MHC region containing the Q region of class I	59		AF111103	±1.0	+4	-2	NEW
NGF-inducible protein TIS21 (aka BTG2)	60		M64292	+2	+2	±1.0	NEW
Ndr1	61		U60593	+2	+8	±1.0	NEW
Gly96	62		X67644	+2	+3	±1.0	+2
p8 protein	63		AF131196	+2	+4	±1.0	+5

MEF & NSC -/- opposite differential expression							
Adrenomedullin precursor	64		U77630	±1.0	**OFF**	±1.0	**NEW**
Fibroblast growth factor	65		M65053	±1.0	**-3**	-2	**+2**
Serum inducible kinase (SNK)	66		M96163	±1.0	**-3**	+2	**NEW**
Annexin VI	67		X13460	±1.0	**-2**	±1.0	**NEW**
Annexin I	68		X07486	-2	**-1.5**	+2	**+10**
Annexin II	69		D10024	±1.0	**-4**	+2	**+2**
AP-2 transcription factor	70		X57012	±1.0	**OFF**	±1.0	**+20**
Jun-B	71		J03236	+2	**-4**	±1.0	**NEW**
PAF acetylhydrolase	72		U34277	±1.0	**OFF**	±1.0	**+12**
Phosphomannomutase	73		AF007267	±1.0	**+3**	-2	**-3**
Sodium/potassium ATPase beta subunit	74		X61433	+3	**+8**	-2	**-12**
Thioredoxin	75		X77585	±1.0	**+1.5**	+2	**-3**
Spermidine synthase	76		L19311	±1.0	**+2**	±1.0	**-2**
Aldehyde dehydrogenase II	77		M74570	+2	**NEW**	±1.0	**OFF**
Voltage dependent anion channel 2	78		U30838	+2	**+2**	±1.0	**-2**
Tenascin	79		D90343	±1.0	**-5**	+2	**+4**
ADAMTS1	80		D67076	-2	**-2**	±1.0	**+2**
Tissue inhibitor of metalloproteinase-2	81		M93954	±1.0	**-2**	±1.0	**+3**
Integrin beta-5	82		AF022110	±1.0	**-3**	±1.0	**+1.5**
Thrombospondin 2 (THBS2)	83		L07803	±1.0	**-6**	±1.0	**NEW**
Membrane glycoprotein M6=major CNS myelin protein PLP/DM20 homolog	84		S65735	+2	**NEW**	±1.0	**-4**
Gelsolin	85		J04953	±1.0	**-2**	±1.0	**NEW**
Gag=antigen LEC-A, env	86		S74315	±1.0	**-2**	±1.0	**+5**
NSC only	87						
Quaking type I (QKI)	88		U44940	±1.0	±1.0	±1.0	**-1.5**

mSin3B	89		L38622	±1.0	**±1.0**	±1.0	**+2**
Retinoblastoma susceptibility protein (pp105 Rb)	90		M26391	±1.0	**±1.0**	±1.0	**+1.5**
Heat shock protein (hsp-E7I)	91		L40406	±1.0	±1.0	±1.0	**+2**
Aspartyl protease 1	92		AF216310	±1.0	±1.0	±1.0	**+10**
Placental growth factor-1 (p1GF)	93		X80171	±1.0	±1.0	±1.0	**+3**
Growth/differentiation factor 1 (GDF-1)	94		M62301	±1.0	**X**	±1.0	**OFF**
Calgizzarin/ S100A11	95		U41341	±1.0	±1.0	+2	**+15**
Cyr61	96		M32490	+2	±1.0	±1.0	**+25**
ADP-ribosylation factor-directed GTPase activating protein isoform b (Shag1)	97		AF075462	±1.0	**X**	-2	**-2**
Camk-2 mRNA for Ca2+/calmodulin dependent protein kinase	98		X63615	±1.0	±1.0	±1.0	**-10**
MAPKAPK5 mitogen-activated protein kinase-activated protein kinase	99		AF039840	-2	**±1.0**	-2	**-2**
Fyn proto-oncogene encoding p59fyn	100		M27266	±1.0	**±1.0**	±1.0	**-2**
Beta 1,4N-acetylgalactosaminyltransferase	101		L25885	±1.0	**X**	±1.0	**+1.5**
Muscle glycogen phosphorylase (Pygm)	102		AF124787	±1.0	**X**	+2	**OFF**
Protein phosphatase 1 binding protein PTG	103		U89924	±1.0	**X**	±1.0	**-4**
Argininosuccinate synthetase (Ass)	104		M31690	+2	**X**	±1.0	**NEW**
Phospholipid hydroperoxide glutathione peroxidase (Gpx4)	105		AF045769	±1.0	±1.0	±1.0	**+5**
GABA transporter (GAT4)	106		L04662	±1.0	±1.0	-2	**OFF**
Sodium bicarbonate cotransporter NBC1	107		AF141934	-3	**X**	-3	**-2**
Glial fibrillary acidic protein (GFAP)	108		K01347	O	O	O	**NEW**
Tropomodulin	109		S76831	±1.0	**X**	±1.0	**NEW**
Cysteine-rich glycoprotein SPARC	110		X04017	±1.0	**±1.0**	-2	**+10**
DSD-1-proteoglycan	111		AJ133130	±1.0	±1.0	±1.0	**OFF**
Extracellular matrix associated protein (Sc1)	112		U64827	±1.0	**X**	±1.0	**-1.5**
Membrane-type 2 matrix metalloproteinase	113		D86332	O	O	-2	**-8**
Astrotactin	114		U48797	O	O	-2	**-10**

Adipose differentiation related protein (ADRP)	115		M93275	±1.0	X	+2	NEW
Ventral neuron-specific protein 1 NOVA1	116		AF232828	O	±1.0	±1.0	OFF
Neuronal pentraxin 1 (NPTX1)	117		U62021	-2	X	±1.0	-10
Receptor activity modifying protein 1 (Ramp1)	118		AF209904	±1.0	±1.0	+2	-7
Lunatic fringe	119		AF015768	±1.0	X	±1.0	-4
TPA-induced TIS11	120		X14678	+2	±1.0	±1.0	+6
ITM2B - E25B protein Integral Membrane Protein 2B	121		U76253	O	O	+6	NEW
NMB	122		aj251685	-4	X	±1.0	NEW
B-cell translocation gene-1 protein (BTG1)	123		L16846	±1.0	+1.0	±1.0	+2
MEF only							
Keratinocyte growth factor/fibroblast growth factor-7	124		U58503	±1.0	-10	+3	X
NOV protein	125		Y09257	+2	OFF	±1.0	X
TGF-beta binding protein-2	126		AF004874	±1.0	-4	+2	X
GATA-6=zinc finger transcription factor	127		S82462	±1.0	+4	±1.0	X
PDGF-alpha-receptor (PDGF-alpha-R)	128		M84607	-2	-6	±1.0	X
Vascular smooth muscle alpha-actin	129		X13297	±1.0	-6	±1.0	±1.0
Alpha-2 collagen VI	130		X65582	±1.0	-8	±1.0	X
Laminin alpha 4 chain	131		U69176	±1.0	-4	O	O
PGI (biglycan)	132		X53928	±1.0	-5	+2	X
Thrombospondin 1	133		M87276	-2	-4	+2	X
Fragile X mental retardation syndrome protein (Fmr1)	134		L23971	±1.0	+3	±1.0	X
Osf-2 for osteoblast specific factor 2	135		D13664	±1.0	-20	±1.0	X
Ndr2	136		AB033921	±1.0	+10	+2	±1.0
P53	137		X00741				±1.0

Tuberin (Tsc2)	138		U37775	±1.0	**X**	-2	**OFF**
Alpha glucosidase II alpha subunit	139		U92793	±1.0	±1.0	±1.0	+
DAN	140		D50263	±1.0	**Q**	±1.0	**+3**
intracisternal A-particle element	141		D49812	±1.0	±1.0	+2	**+5**
Annexin V	142		U29396				**+1.5**

Key=
New=de novo expression
Bold= gene was confirmed in that job
+1.0 = no difference
X = no poison
Q = in process
p = partial poison
O = no band

Below follows additional discussion of nucleic acid sequences whose expression is differentially regulated.

TSC1

TSC1 is a novel 2520 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

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1 GGCTCTGGCTCGGGCTCGGGCTGGGGCTGGGGCTTGGGCTCCAGCTCGGGCCCTGCACCTGTGACTCGGCGGCGTTGCTC 81
CTCCGCTGCCCCATGGCCCCGTCCCGGCTGCAGCTCGGCCTCCGCGCCGCCTACTCCGGCTTCAGCTCGGTAGCCGGCTT 161
CTCCATCTTCTTCGTCTGGACGGTGGTCTACCGACAACCGGGGACTGCGGCGATGGGGGGTCTCGCAGGTGTCCTGGCAC 241
TGTGGGTCTTGGTGACTCACGTGATGTACATGCAGGATTACTGGAGGACCTGGCTCAGAGGGCTGCGCGGCTTCTTCTTC 321
GTGGGTGCTCTCTTCTCGGCAGTCTCCGTTTCCGCCTTCTGCACCTTCCTGGCATTGGCCATCACCCAGCATCAGAGTCT 401
CAAAGACCCGAACAGCTACTACCTCTCCTGTGTCTGGAGCTTCATTTCCTTCAAGTGGGCCTTCCTACTTAGCCTCTACG 481
CCCACCGCTACCGGGCTGACTTTGCGGACATCAGCATCCTTAGTGATTTCTAACCCAGGGAATGAGGTCACCACAGCCTG 561
GGGGCCCTCGGGATCTGGACTCAGCTTCCGAGTCAGCAAGGGAGCTCACCCCAACCCCTGGGGAACTCCAGAACCATGGC 641
AGAGTATATGGGCCCGTTCAGTTTCTCAGAAATCTGTCTGGTCCCCTTTTGGGGAAGATATAGAGCTGTTAAAGGGATAC 721
TGCCAATCTGCCCAATCTGCCCGTTAGCCCAGCTAGAGGGCAGCTTAGACCTTTCCAAATAGATCTATTTTCTTAGCCCT 801
CTGAGGGATCTCTGTAAGTAGGGCCACGACAATGAATTCAATGGGTAGGATTGGAACTATGGCTAGTGACAGGGGCTGGG 881
ACAGGCTTCCTTGCTACCCCAGACTTCATTGAAGCTGTGTGTGGGGGAGGCATCAAAGGTCTGGTCAAGAGAGGAATCTT 961
TAGTACAGATCTCCATCCCCTGTTCCCCACCCTGTTACCCTGAAGTGTCGGGTAGCCAAACTCACCGGTCCTTAGGGAAT 1041
TGACAATTGGCTCCTTCCCTAAGCAGCACAGTTGGACAGAATCCAGCGTCCGTCCGTCCTACCTTCCCATCCAGAGTTTG 1121
TTTCCCATGAGGGTGCTAGCGCCAGCCAACCATTCCCATGTGTCGCATATGCACACATGACCACACACACCAGAGCAGGA 1201
CTCCTCGGATGAGGCTAGACTTGAGGACCACAGGAAACACACCCCTGCACTTAGAAGGGCTTTGGGATCGGGGGCAACCT 1281
GGTGGGGGCAAGTGGGAGCTCTCCATCTGTACTGAGTCTCCAACCTTGCCCCTCACTGCACAAGACCACCCTGACCGTGA 1361
GGACCTCCTCCCTGCACCAGATCCTAACTCTGACCTTTCACCTTCTCTCTCTCCTGAAGGAACTCTTCTGAGTGGACATG 1441
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GGCCCAAGGCCTTACCTAAGCGGAGAGGGAGGGCAGGGGCTGCTACTCTTCTCTGTAAC CTTCTCTGATGGGTTGTCACT 1521

TTGCACGTCTACTCTTCCACTTGGGCACTGCCCCCAGCTCTCTGCCTTACCTGTGTTATGGGCACTTAAGCAGAAATACA 1601

GCGGCCATTTTAACCAGCAAAAAAAAAAAAAAAATAGGGGGGTGGGCGGTTTTGAGAGGGGACAAGAGTGGGCAAGATGGG 1681

GGCTCTAGCTGTCTGATCATCTCCCTAAGTTTGGGGCTACTAGACGGTATTCCTCATCTCTGGTCCCCTATGGGAGACCA 1761

CCAGCTGAGATCTCCTTTGCTCTCCCAGTTCTGTCCCAGCCAGGGTTAGGATGCCCACAGACTCAACATCCCTGCAGATT 1841

CCATCTCCCCACCCTAAGCCAAGGTAGATGGGAAAGGGAATCTTTCTTTTTCTACCCCAGCCAGACTACTTGGGGCTCCA 1921

AGTTGACCAGGATGTGTGGATTCAGAAGCAGAAAGGCAGGAGCTAGCACCTCTCTCACGCTGGGTACACTTGTCCTGGCC 2001

TGTGTTTGCCTCACCCTGGCCTTTACAGTGTAAAAACACCATGGGACTTTAGAGCAGGGAAGGATAAGGAACAGTGTCAC 2081

TTCTAGAGCCTTCTGCTGGTAGACGCTCCTACTGATAGAGGAGGTAAAGACTACTGACCTCCCGGCTAGGCCTGGCTTAA 2161

GCCAGGCGTGGCCTGCGTCACAACCTTTTGCGGTGTCTTAGCAACCTGAACCTGAGATCTTATTCCCGAATCCCACAGGG 2241

CCCAATGTGCAGGGCTCAGCCTGGGGCCATCTCCCTTTTCACCTGGGTTGGTGAGCATGTATTTGGAGTGGTTTCTTCCT 2321

GCATGTATTAGCCAAGGAAGGACAAGGGACTAGAGGGTCTGAGTTAGGTCCAGACTTGTCCCCTTTCCCCAGCCCATCAC 2401

AGGATGCTGGGTGCACACCCACTCCACTGACGATGTCCCACCAACATCCAGGAGGCGTTCTCCCAAGGACTTTAAAGCAA 2481

ATAAAACATATATTGTTCAGAAAAAAAAAAAAAAAAAAA (SEQ ID NO:1)

TSC2

TSC2 is a novel 1863 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 AAGCGTGACCCTAAGTCTAGCCTGGAGCCAGGGCTAGAGTGGTCATTTCTTTGTGGGGTGCTGCCAGGGAGGGGCCAGAC 81

CCACAGGCTACTCAAAGGGCCTAGAGACCCCTCCCCAGGCAGGTGCTGCCCCAGGAGGAGCATGTCCTGGGGTCCGGGGA 161

CTGAAGTCCATGTGGCCTCAGCCCCCCACACCCAGAACACCGCTTGCCTAAGGTGCTTTTGGCTTTAGTGTGTGATGTTT 241

GCTGTGCTTCTGGGCTGAATTAGCTTCCAAATCAGGACCTGGAGCCTCTACCCTGGCCCAGCCAGCCAGTGTGAGCTCTG 321

GTCTGTGAGATGGGCAGCTACGGGCCAGTGGAGCAGCATGTGGTGGGAGGGGCAAGGCTGGGACCCAGTGGTTTACAGAC 401

CTGTGGCCCTCCTGGAGCAACCTGGCAGCTACGGATCCCAGAACCCCCTGGGCTTCAGCTCCCCCAGAGGGGAGAGGCTC 481

CACGTTGCTTTCCTTCCCCAAAATCCCTTTCTTTGTGCTGGTGTCTGGGACCAAAAGGAGTGGGCAGAGGACTCGGAGGG 561

CCTAGGGGTCCCAGTCGGGGCATCTGTAGCTCCTAAGCACGACAAGCATCAGTGCAGGGGACCCTGGCCTTGACTCCAAC 641

TGGCCTGGCGCCAGGAACCTCCAGGGCCAGAGCAGCCCAGCTGCAGCCAGCCTGCCCACTATGGGTATGTTCCTGGCCTA 721

AGGTCCGGAGGGAGGTTTGGGGTATCCCTGCCTGGGTGCCTGGGTGTGCCCTGGGGCCTCTCAGAAGCACAAATGCTGCC 801

CCCTGGCCGTGAGCAGGCCACAAGGTGAATGTATATAGCATGAGAGGCGGGCACTGCCCAGACGTGGCTGTGAACTTGTG 881

CTGTCTCGGGAGTCCTGACCTTCTGTGCGTGAGTGCCCCCATCTGTGACGTTTCACTCACCGAGGCTGAAGAAAGGAAGC 961

AGGGGAAATGAAAGCAGGGGTTTCTCGCCCTGACCCCTGCGGAGGAGACGGCTCCTACCACTGCGGTTGGCTTCATTTCG 1041

TTTTCCTGATTTCTGGGGTGCCACTTACCTACTCAATCCCAGTGGTCCACCCCCACATCCCCAGGGAGTGAGCAGTCCAG 1121

TGCCAGCTGCCTGTGATTGGTCCCCAGTCCCTATTACCCAAGGGGACCCTACAGCTCTGGTGGGTAACAAGGAGGGCTAA 1201

GCCACCAAACCAGAGCCCGATCCCTTGCCGAGCCAGGAGGAGGGATCTGGCTGAGAAAACTGATAGGACTGGAGGCCCCC 1281

ACCCCAACCAACACTCTCTGGTTTATGTGAGTAGCAGAAGATCCCGGCCTGGAGCATCCTTCAAGCCCTTCTCCCTGTGC 1361

CCACCCCGCCCCCCCCCCCCCCCATATCACTATGCAATTCTTGACCCCAGCTCCAAAGCTTGCCCTACCCGGTCCCAGCT 1441

CTGTCCGGCCCAGAAGGTGGCTAGCTGGTGGGCCACAGGTGACCAGGGTCTCTTTGTTT TTCATCACAGCGGTGGTGTGC 1521

CGCACCCTTCCTCCCATATGTGATTTTGTGAGATTGCCTCCCAGTTACGGTCCCTCTGCCTGCATCTGCCCCCAGTGGAC 1601

TATGTCATCTGAATCGAGCCAGCCCCAAGTTCCCCTCCAGCCTCTGTAGGGCCATGGCTGTGTGTTACTGTTGCTGTGCT 1681

TTCATTTTTTAAACTGGGTTTGGGGTTTGATTTTTATTTCTGTGGGGAACTTTATTTTTCTTGGCAAATAACTAAAGTTC 1761

TTGTCCATGTAATTTCTGTGGTCTCTATTCAGCTTGGGTTTCATGTTTTAAAATAAACAATTTTAAGAAACAAAAAAAAA

1841 AAAAAAAAAAAAAAAAAAGC (SEQ ID NO:2)

TSC3

TSC3 is a novel 750 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

CTTGTTTATCCTACTCGGGTAGTTTCCTACTAATTTCAAGACTAGTGTTAACATTCTAAGGTAGTTATCTTAGGGTAGAT 81

TCAAGGTTTTAGATGACTAACAGTTCAGATTTTCTGATCAATTTTTTAAACACTAGAGAATAAAAGTGTACTAGAGAATA 161

AAAGCAGCTTCATAGTTAATTCTCACCAATTGGCCCTTTGCTAGCTGCTGGCTTTAGGTACACATAGGATAATATGTGTC 241

CACGTTTCTACTTGGAACTGGTAAAAGTTGTCACTGGCTGGAAAATGGTATCTCTCTCTTGTATACAAGATGGTCCATTG 321

ACACTGGTACTTTATGAAGCAGTTCTTTGTTTGTTTGATTGAGCTCTCTTGAACCTTGTTCATCTTTTAGTTTTTGCTTG 401

GAATGGAATGGAACTGGTTTGAAGTTAAAGGAAATATTCATTTTGAAACTTGTTCATTTTGAAAGGAAATGCAAGTTTCA 481

AAATGAAAAATAAAATGAAAAAGGAAATAAATTATTGTCCCAGATGGTCACTTGAGTTTTAAAAAATGGCTGCACACAGT

561 AAAACTGCTAAAAACAAAAACTTACCTCATTATTGGTTTGCATCTTTTTTCAGCTACTAATTTTATACCAAAATGTTAAA

641 TATTTATATTGTTTGAGTTTCAATCTTGTATGGAAAAAAATAATTAGTAGGTCTAAAAATGCCATGCTTTCCAATAAAGA

721 AGTTAAAAAAATCATCAGTAATGTGAATTT (SEQ ID NO:3)

TSC4

TSC4 is a novel 281 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 GGGCCCCTCCGTCTCAGAGCAACTATACCCTCTACCTCGGAAGGAGCAGCAGAGAGAGAAGCCACAGGCCACCAGGAGGC 81

CCAGCAAAGCCACCAACTATGGAAGCTTCTCAGCCACCCCACCTCCCACCCTCTGGGAGGTCAGCACAAGAGTTGTGGGC 161

ACAAGCCGTTTCCGGGACAACCGGACAGACAAACGGGAACATGGCCATCAGGACCCAAATGTGGTGCCAGGTCCTCACAA 241

GCCAGTAAAGGGGAAGCTGCCCAAAAAGAAGGACAGAATTC (SEQ ID NO:4)

TSC5

TSC5 is a novel 1568 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 CGCGCGGGAGCCAAGATGCCTCGCGGGGACTCGGAGCAGGTGCGCTACTGCGCGCGCTTCTCCTATCTTTGGCTCAAGTT 81

CTCTCTCATCATCTACTCCACCGTGTTCTGGCTGATTGGGGGCCTGGTCCTGTCAGTGGGGATCTACGCAGAGGCAGAGC 161

GGCAGAAATACAAAACCCTGGAAGAGTGCCTTCCTGGCCCCCGCCATCATCCTCATCCTCCTGGGGGTGGTCATGTTCAT 241

CGTCTCCTTCATCGGGGTGCTGGCTTCCCTCCGGGACAACCTGTGCCTTCTGCAGTCGTTTATGTATATCCTGGGGATCT 321

GCCTGGTCATGGAGCTTATTGGTGGGTCTGTATTTAGGGGCCGCCGGAACCAGACTATTGACTTTCTGAACGACAACATC 401

CGGAGAGGAATCGAGAATTACTACGATGATCTGGACTTCAAGAACATCATGGACTTTGTTCAGAAGAAGTTCAAGTGCTG 481

TGGCGGGGAGGACTACAGAGACTGGAGCAAAAACCAGTACCATGACTGCAGCGCCCCCGGGCCCCTGGCTGACGGGGTTC 561

CCTACACCTGCTGCATCAGGAACACGATGTTGTCAACACCATGTGTGGCTACAAAACAATCGACAAGGAGCGCCTGAATG 641

CACAGAACATCATTCACGTGCGGGGCTGCACCAACGCCGTGTTGATATGGTTCATGGACAACTATACCATCATGGCGGGC 721

CTTTTACTGGGCATCCTGCTTCCTCAGTTTCTTGGTGTGCTGCTGACCCTACTGTACATCACCCGTGTGGAGGACATTAT 801

CTTGGAGCACTCTGTCACGGATGGATTGCTGGGACCTGGTGCCAAGTCCAGAACGGACACAGCAGGCACTGGATGCTGCC 881

TGTGCTATCCCGATTAGCTATGCTGATTGAGCTATCCTGGCCCGGCACAGCAGCTCCCAGCCGGACTGTACTGCAAAGTG 961

CATCTAAGACTACACAAGCTGGACAGGACCAGCTGCAGCTCCTCTGCCCACCCACGGCGCTGACCAAAGCCCAGGGTGTA 1041

TGTACCTGCGTATAGTGTCTGATGGCCACTCCTCCTAGGGGAAAGCTGAACCCTGTGGGATCCCGGGAACAGGGATAGCC 1121

CAGCTCCGGTTCTGAGTCCTGGAGAAGGCAGCTCAGGGCTCCGTGTGGGCTCTTTTTCTTTCTGGCAGTGCCTTGGCCAG 1201

TGGTCATTATGCCCCTTCAAGGGCAGTTTTGCAGTGATTATTTTTAAAGGCAAGAAGGGAGTGTATCTGTTCTATAGGGA 1281

AGTCCTGGGTGCAGCCCTGGTACACTACTCTAGATGTGACGTTGGACTGTGTCTCAAATTCCCAGGTGCCTTGAGTCCTC 1361

TGTAAGGCTCCTGCTTTGCCCACCCATTTTCTACATATGTTTTTTTTCTTTTTTTTTTTTAATAACCGTGTTTTGTATAC 1441

AATTAACAAGAGTTTCTGGCTATTCAAAACTAGCCACCCCTGACCGAGTCCACTCACCC CTCCCCGTTAGTTCATTAATT 1521

GAACAATAAATATGTGTTTTGGGGGGTGGTCTTTAAAAAAAAAAAAAA (SEQ ID NO:5)

TSC6

TSC6 is a novel 300 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
  1 gccggctctt tgtggaggac tccatccatg accagtttgt gcagaaagtg gtggaggaag
 61 tagggaagat gaaaatcggc gacccccctgg acagggatac caaccatggc ccgcagaacc
121 atgaggccca cctgaggaag ctggtggagt attgccaacg tggtgtgaag gaaggggcca
181 cactggtctg tggtgggaac caagtcccaa ggccaggctt cttctttcag ccaaccgttt
241 tcacagacgt ggaggaccac atgtacatcg ctaaggagga gtccttcggg cccatcatga (SEQ ID NO:6)
```

TSC7

TSC7 is a novel 965 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 CCCACAGCTCCTGCCCACTCACCAGGTCCAGGGGAGAGCAGGCGGTGACTCGATGACAAGTGCCTTTAGTTGAAGAGCAC 81

ATCTCACTCATTCCTCTCTCAGTACCTGATACATTCCTCTGTGCTAACCCCCCCCTTGGGGAGGACCCACCCTCTGGAGGC 161

TGGACTTGGGGCGAACAGGCACTCACCTGTCACTGCCAAGGGCGGGCAGGCCATCCTTCCGAGCCCATGGGAGCCGGGAC 241

CACTAAGACTGCTGGTGGGAAGAAGTTGGGTGCTGGGCTGATGGTCTTGCTTTCTCTTGGTCTTCGCTTGTAATGTGGCT 321

GGCCCATGTTGGTTTTATGTTTAATGCTGTGCTTATAATAAGAAAGAGCCCCCCCCAAGCTGTACATTTATAAAAAGTGAT 401

CATATACTGTATATAGAAAAATCTAGAAGCACATATGAATGCAGCAGGTAGTATTCCACTGTACCCATTCATGAAGGTAG 481

GTTTTATTACAGGACTCGCACCAGGTACTTACAGACGCGCCCTCTCCTCTTTGCCTAGAGAAACAGTCACTGCATTCCCG 561

CACAGTCCCTCAGACCCCCTTACCCTCTTCCCTGTAGGAAATTCTCCTGTGACCCCTCTGCCGTCCTCCCTTACTTCCTA 641

```
AATAAATGTAACGGAGTCAGTGCAAAAAAAAAAAAAATAAATGACATTTATTGTGGGTTATAATTTTCTCCTAAAAACAAA
                                                                                721
ACCAGTGGTATGGTCATACCCACCATTGTTTCCCCACTTTCCATGACCGTCACAAACATCTGGGATGAGCACCTTGTGAG 801
CAGGAAAAGTTATGCTTTAAGAAATTTCTGGCCAGGCGTGGTGGCATACACCTTTAATCCCAGCACTCGGGAGGCAGAGG 881
CAGGTGGATTTCTGAGTTCGAGGCCAGCCTGGTCTACAAAGTGAGTTCCAGGACAGCCAGGGCTACACAGAGAAACCCTG 961
TCTCG (SEQ ID NO:7)
```

TSC8

TSC8 is a novel 408 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
  1 gccgggtctg aaaaggacta ggctggcatt ggtgacaccg agcttgttgg cagccacaca
 61 ggtatagttg ccatagtgtt cctcagtgac attggtcacc gtcagggagg actggccctc
121 agtgctctta atctcaaggc catttgcact gtttatcctg gtgtcatccc ggtaccactc
181 aaagtcaggt gcaggcaccg ctgaggcttc acatttgagg gaagcttgtc gtcctgtggt
241 ggcttcgttg ctcttcgact ccgtgatagt gggtggatag ttcacagtga ccttgacttg
301 tttgacatcc gccgaggaga cctcgttggc agccttgcac tcatatttgc ctgactgttc
361 cctggtgatg cctaggatct ccagatattc ttcttctcct tcaaatty  (SEQ ID NO:8)
```

TSC10

TSC10 is a novel 354 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
  1 gtgcaccaga tgttctacga ggccctagat aagtacggga acctcagtgc tctgggcttc
 61 aagcgcaagg acaagtggga gcgtatctct tactgccagt actacctgat tgcacgcaaa
121 gtagccaaag gcttcttgaa gctcggccta gagcgtgccc acagcgtggc gatccttggc
181 ttcaactctc cagaatggtt cttctctgca gtgggcacag tgttcgcagg gggcattgtc
241 actggcatct acaccaccag ctccccggag gcctgccagt acatctctca tgactgccga
301 gccaatgtca tcgtggttga cacacagaag cagctggaaa agatcctgaa gatct (SEQ ID NO:9)
```

TSC11

TSC11 is a novel 955 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
1 CGGATCATCTGGGTCGCGACCTTGAGGCCGGGAATCGAGTTTCCAAACGTGCGGGGGCCTTCGCCGGCTCTGCTGCCCCC 81
TTTCTCTCCATGGCAGCGGCCCGGAACCTGCGCACCGCGTCATATTCGGAGGCTTCATCTCCATGGTCGGCGCCGCCTTC 161
TATCCCATCTACTTCCGGCCCCTTATGCGGCTGGAGGAATACCAGAAGGAGCAGGCTGTAAATCGAGCTGGTATTGTCCA 241
GGAAGATGTGCAACCGCCAGGTTGAAAGTGTGGTCTGATCCATTTGGCAGGAAATGAGGCTGTCAGCAAGTCTGATGAGG 321
AAAGTGGACGTCTTTATCCTGTGCACTCCGCAGTGGGGACAATAGATGCCTCACTGTGGCAGCATGGCATGGAGAGGGAA 401
CTCTCATGCTGCTAGCCAGACCCCTTGTGATAGAGACTGTGTGCAAAGACAGTGCTTCCCTTAACTCCCTGGAGAACCTG 481
AACAGATGCCACCATTAGGAAGTGCCTTGCGGCTCCATTGACTTTGCAGGAGCAGAGCCAGCCTGCAAGGCTGTTTGTGG 561
AAGATCTGCTGCTCCTGCAGTCTTTATCACTTCCAAGCTGTGATGTGAACACAAGCAACCTGTGGGCTCAAGGTCCGTGG 641
CTGCTCTGACACCTTTTGAATAAGCGATTTCAGTGCAAATGGCCTTGCCAAGCTGCCTCGCAGGGTTCTTGGAGGATGTT 721
TCAGTTGATAAAACTGTTTGAAGACAGGATCCTTGGCACTGTTTAAGAATATACACTGCTCAGCTTAACCATTTCATTGA 801
AAGTCACTGTGTGTGGAAGTGAATAGGGAGCGAGTCACACTAGACTATACCACACACAGTAGATTCCTGCGTGAGGCTGC 881
```

AGGTATTAAAATGGTTTCTCTTAAAAAAAAAAAAAAAA (SEQ ID NO:10)

TSC12

TSC12 is a novel 1113 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 GGAGACCCAAGATCTGAACCAGCCAGCCAGGTGCTGCACAGCCTCAACTTTGGGAGCAGAGGCCCTGTGGGGTTAACTTG 81
GGTCTGCCAGAAACAGTGCTTCCCGCAGGGAAAATCTTGGGTCAAGATGGAGGCTGCTCTGGAACACTGAGTGTTTCAAG 161
GGAGAAAGAGTGGGAACCGTGGCCCTTTGGGGCCAGACCCTGCAGGAGCTTGCCTCGCCTTTGAGGAGGAGGCACTGCTC 241
TTCAGGTGCCCTGGAGGGGCTTTTAGTGCCATCCCCACAGCAGAGTAAAGGTGGCGCGTATGTCATCGGGTGGCTTTGCG 321
CTGGTAGAACGCTGTTCTCTACCCTGCTGCAGCCTTTCACACTCACACACACCCAAACACACACTTCTCGGCCCTGTATG 401
TTCAGGTGAGAGACAAGGGAAGATGGCTCATCATTTTCAGCCATGTCCCCAAAGTGGCCTCTCTTTCATGCTCTGTGGGC 481
TTTGGCCTGCAGCTGTTCCAGAGTTAGGGATGTGATTTTTGTCTGTGAGGTACCCCTTGCCCTAGTGGATCAGTTACAGG 561
CCTATGTCCAGCACCAGAGTCCCTGTTCCGATATCATCACAGATAGCCTGTTGTTTTCCACAGAGGAGCCAGATGTAAGT 641
CAGACACCTCCAGCCTACCAGTCTCCTGCCATCAGCTTTGGCTCTAATGGGCTCTTGGTGGCCTCCTTGGTGTGTCACTG 721
GTACAGGACAGCAAGTGGCTCAGAAAGGCTGCTTGCTCCTGAGCTCAGCCACTTATTCACATGGTTCAGAGCAGATCTTT 801
GTACTCTTCAGACTCAAGTATGGTGATCTGTTTGACAGTAGAGGTCTGGCCTACCCCTCACCCTCATTCTCCAGCACCTC 881
TAACAAGAACCACACTCATGCCTCTGGTGTCAGTTTTCTTGTCTGCCTTCCCTGGCCTACCTAGATATTTATTTCTTGTG 961
TTTTATGAATAGTTAAGCCCTGCCCATCTGTGCCTTTCAGACGGAAACACAGAAACCTAGGCTGTGCCATTTGTCTTCTC 1041
ACAGTTGTTTAATGAAACCTCAAGGAATATGGAAATAAAGCCTAGACCCTGGAGTGGTGAAAGAGTAAAAAAA (SEQ ID NO:11)

TSC15

TSC15 is a novel 594 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 AGATCTCTGTTTCCTCTTTCTTCTCTCCTCTATGCTCTTCTGTAGCCTACCCTCAGGGTGATCTCTAACCCAAACTAATC 81
CCGAGGAACAGACACTTGGCTCAGCTCCACCTACTACCTGGCTCACCTGTTCCCAGAATCTCCATAGAAGAGGGCACTTT 161
CTTTCTCAAGTTACCCTAACATTCTCTGCAGGATAAAATCATGAGTCCAGCCTGTCTGTGGAACTGGGGCCTGTCTGCAG 241
CTTCCCTGCAGAAGTGTCCATTCACTTTGGGTGATCTTCCCGACCAAGATACTTAGGTGTTTTGGCCAGCACCAGTATTT 321
CTATGAATTCCTGATCTGGAGTTGAATAGACAGGAATCAAGACCTAGGCTTTTCACTGTGTGAACCTGAGCATGTGGCCT 401
GACCTGCTGGAAGCTCCTCTGCTCTTGTGTGAAGCAGGAATGCTGTCAGGCACACAGCACAACACACCAGTGGTGGAGAA 481
CGCTAATCCCAACACACAAATTCCACAGAAATGGCACTATCCTCGGGTCTCCTGCCTAACCATGGACAAAGCTGAGAATA 561
AACAGTGCTTTACTTTGAAAAAAAAAAAAAAAAA (SEQ ID NO:12)

TSC16

TSC16 is a novel 713 bp gene fragment. The nucleic acid was initially identified in a

cloned fragment having the following sequence:

```
1 CAATTGTTTTTTCTAACCATCTTAGGGAACAATACATTGCAATAATTGATAATAGTGCCATCACTGTAATAAACTTTAGA 81
GACTTTTTTTAATGTAAAAGTTGTTGGTCACCTTGTTTCCTGTAACCTTCACTCTGTCACACGAGTTGGCTCATAGGTTG 161
TGTTTGTCTATCAGAAATAAGAAAAACACAAGTGAAGAAAATGTTGGCATGAAGTCATCCATCTGCAATGAAAAACCTAA
241 AAGACTACGGGTCACTCATGTTATCAATATAATTTATAATCCTGTTCAGTGTACAAAATTGTGGGTTTTGTACTCACCCA
321 AAAGACTAAAACACCAGTTTTTCTTACAGTATCTATCTACAGAGCTTATTCTCCCCTATTATTTGGGAAACTCTGAGACT
401 CCATATTGCAGAAGTCAAGGAATAGGCCATATAAGAAAATGTAGCTTGTTTTTATTATTTCTGCATATTTATTTCTAGAT
481 CTTGGGCTCATTTGTTAACAGAATAAGTTGTCAAAGGTAAAGTCCTTGAGTCTGGGAATGAGCCATCGTTCCAAAACCAA
561 CACACCCTGTGTGGAAATTTTACTTGACTCTGTTTTGCTGCATAGAATTCAGTGTCTCTTGGCCATTCCCCCTCATTCCT 641
ATACTAAATTCTTTGAAGACACTGGTAACAGTTTGTGGTAGACTACAGTTGAAAAAACTCAATCCTTATTTCT  (SEQ ID NO:13)
```

TSC17

TSC17 is a novel 306 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
  1 ggatccctcc accctatgac aagaaaaagc ggatggtggt ccctgctgct ctcaagggtt
 61 gttcgcgctg aagcctacca gaaagtttgc ttacctgggg cgtctggcgc atgaggtcgg
121 gtggaagtac caggcagtga cagccactct ggaggagaaa cggaaggaaa aggccaagat
181 gcactatcgg aagaagaagc agatcttgag gttacggaaa caggcagaaa agaatgtgga
241 gaagaaaatc tgcaagttca cagaggtcct caagaccaac ggactcctgg tgtgaaccca
301 ataaag (SEQ ID NO:14)
```

TSC18

TSC18 is a novel 66 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
  1 gaattcgaat cacgctcacc agccgcaacg tgaagtcgct ggagaaggtt tgtgcggact
 61 tgatca (SEQ ID NO:15)
```

TSC19

TSC19 is a novel 1613 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
1 CCAGCTCAGAGGTTCTAGGGGCAGCCGGCGCGCTTCTCTAGTTGCAGCTTGGGCGGCTCCTGTGGTGGGCGGCTAGGGGC 81
GAGCCGGGATGGGCTATAGACGCGCGACGTGATCAGTTCGCACGCGGACCCACGCCTCCCATCGCTCTGCCTCAAGAGCC 161
TATTCTGTGGGTGCAGGCACGCACCGGACGCAGACCCGGCCGGAGCATGCGGGGTGCGGTGTGGGCGGCCCGGAGGCGCG 241
CGGGGCAGCAGTGGCCTCGGTCCCCGGGCCCTGGGCCGGGTCCGCCCCCGCCGCCACCGCTGCTGTTGCTGCTACTACTG 321
CTGCTGGGCGGCGCGAGCGCTCAGTACTCCAGCGACCTGTGCAGCTGGAAGGGGAGTGGGCTCACCCGAGAGGCACGCAG 401
CAAGGAGGTGGAGCAGGTGTACCTGCGCTGCTCCGCAGGCTCTGTGGAGTGGATGTACCCAACTGGGGCGCTCATTGTTA 481
```

ACTACGGGCCCAACACCTTCTCACCTGCCCAGAACTTGACTGTGTGCATCAAGCCTTTCAGGCACTCCTCTGGAGCCAAT 561

ATTTATTTGGAAAAAACTGGAGAACTAAGACTGTTGGTGCGGGACATCAGAGGTGAGCCTGGCCAAGTGCAGTGCTTCAG 641

CCTGGAGCAGGGAGGCTTATTTGTGGAGGCGACACCCCAACAGGACATCAGCAGAAGGACCACAGGCTTCCAGTATGAGC 721

TGATGAGTGGGCAGAGGGGACTGGACCTGCACGTGCTGTCTGCCCCCTGTCGGCCTTGCAGTGACACTGAGGTCCTCCTT 801

GCCATCTGTACCAGTGACTTTGTTGTCCGAGGCTTCATTGAGGACGTCACACATGTACCAGAACAGCAAGTGTCAGTCAT 881

CTACCTGCGGGTGAACAGGCTTCACAGGCAGAAGAGCAGGGTCTTCCAGCCAGCTCCTGAGGACAGTGGCCACTGGCTGG 961

GCCATGTCACAACACTGCTGCAGTGTGGAGTACGACCAGGGCATGGGGAATTCCTCTTCACTGGACATGTGCACTTTGGG 1041

GAGGCACAACTTGGATGTGCCCCACGCTTTAGTGACTTTCAAAGGATGTACAGGAAAGCAGAAGAAATGGGCATAAACCC 1121

CTGTGAAATCAATATGGAGTGACTTGCAGGGTGACACAGTACTGTTGTCCTTCAGATGAGCCATGTTTTGTGGGCTCAGT 1201

CGCTCTATCATATCCTGATAGAGATTGCAGACTGGTGGCATGGGCCCAGCCTGGTGCTAGAACTGGGAAGGTACATGCTG 1281

TTCTGACCCCTTAGGTCCCAGCCAAGGATGCCCTGACCCATTGGAACTGCTGTAAAATGCAAACTAAGTTATTATATTTT 1361

TTTTGTAAAAGAAAAAAAAAAAAAAAAAAGAAAACTCCGCGCACAGGGGGGGTACGTCCCAATTCGCCAAAAACAGATGC 1441

TAGAACCCCTGGCGGCCCCCCCACCCCCACGGGAGACACTAGCTAACCAATTAATGCTT GGAAAATCCCTTCTGCACCGG 1521

TAGTACGAAAGGCCCACGATGCCTTCAAAGCTGCCTGGACGGAATGCAAATGAACGCTAATTTCTAATCCGGTAATTGTA 1601

ACCGCATTCTACA (SEQ ID NO:16)

TSC20

TSC20 is a novel 2245 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 ACGTGACCGTGAGACCCTAGGAGCAATGGCGGGGCGGCTGGCTGGCTTCCTGATGTTGCTGGGGCTCGCGTCGCAGGGGC 81

CCGCGCCGGCATGTGCCGGGAAGATGAAGGTGGTGGAGGAGCCTAACACATTCGGGCTGAATAACCCGTTCTTGCCCCAG 161

GCAAGCCGCCTTCAGCCCAAGAGAGAGCCTTCAGCTGTATCCGGGCCCCTGCATCTCTTCAGACTTGCTGGCAAGTGCTT 241

TAGCCTAGTGGAGTCCACGTACAAGTATGAATTCTGCCCTTTCCACAACGTCACCCAGCACGAGCAGACCTTCCGCTGGA 321

ATGCCTACAGCGGGATCCTTGGCATCTGGCATGAGTGGGAAATCATCAACAATACCTTCAAGGGCATGTGGATGACTGAT 401

GGGGACTCCTGCCACTCCCGGAGCCGGCAGAGCAAGGTGGAGCTCACCTGTGGAAAGATCAACCGACTGGCCCACGTGTC 481

TGAGCCAAGCACCTGTGTCTATGCATTGACATTCGAGACCCCTCTTGTTTGCCATCCCCACTCTTTGTTAGTGTATCCAA 561

CTCTGTCAGAGGCCCTGCAGCAGCGCTGGGACCAGGTGGAACAGGACCTGGCAGATGAACTGATCACACCACAGGGCTAT 641

GAGAAGTTGCTAAGGGTACTTTTTCGAGGATGCCGGCTACTTAAAGGTCCCAGGAGAAACCCATCCCACCCAGCTGGCAG 721

GAGGTTCCAAGGGCCTAGGGCTTGAGACTCTGGACAACTGTAGAAAGGCACATGCAGAGCTGTCACAGGAGGTACAAAGA 801

CTGACGAGTCTGCTGCAACAGCATGGAATCCCCCACACTCAGCCCACAGAAACCACTCACTCTCAGCACCTGGGTCAGCA 881

GCTCCCCATAGGTGCAATCGCAGCAGAGCATCTGCGGAGTGACCCAGGACTACGTGGGAACATCCTGTGAGCAAGGTGGC 961

CACGAAGAATAGAAATATCCTGAGCTTTGAGTGTCCTTTCACAGAGTGAACAAAACTGGTGTGGTGTAGACACGGCTTCT 1041

TTTGGCATATTCTAGATCAGACAGTGTCACTGACAAACAAGAGGGACCTGCTGGCCAGCCTTTGTTGTGCCCAAAGATCC 1121

AGACAAAATAAAGATTCAAAGTTTTAATTAATTCCATACTGATAAAAAATAACTCCATGACTTCTGTAAACCATTGCATA 1201

AATGCTATTGTAAAAAAAATTAAACAAATGTTAACAACTTTAACAATTCACTAAAGTAAATGGTTATGTATTATAAATAT 1281

GACCATCTGGGTTAAGAAGATTCCATTCACATAACATTCTCAACTAATTTCTGAAGAACAAATGAACACAAAGGCTTCCA

1361 TAAGTTAATCCACATGCGCATCCATACTGGGGGAAGGCCTGCCAACCAGGTACACAAGACTCTGACACTACCATATACTG

1441 TTACTATTCAACACTAGAGAGTTAGACGACAACAGGCATCAGGACAGTGGTGGGTCCCA GTTCCTAGACCCATGGCCCCA

1521 CCTCCATTACCCACACACGGGCCTTAAGGCTCTCTCTCCCCTTCTTGGCCCTTCCCACCCAGGGTAGATCCTAGAAGCCT 1601

CAGCTCCTAAGAGGTCTGGAATGGATGGGAAAAGTGGCCCCTTCTGGGACGTTCTTTGGTCCTCCCCTGCACACCTGTCC 1681

TCAGAGCTCAGCCTGATTCCAGAAGAGCAGATGCTCAGGAAAGCTCCCCGCATGGGATGGGACCCAGGGTGCACTACCGC 1761

CTGCCTCCCCAGCCATCACAACAGCCCCAGAACTGCCCAGCCCCAGCCTGGAATGTCAGCCCAGGAGGAGTTAACCAGAG 1841

TAGCTTACATACAATCTAAAGCTTAATGTAACTGTATACAACTTGAAATTGTCCCGATGAGCTATCAATCACAAACACTG

1921

TCCTGTTACCACAGAGACCAAAAGCCTGACATGGGAAACAGTTCATAAATATGAATAAAAATAAACAATCTTAAACCATG

2001

GTAACAGTAGCACCAAATACACATGATCTAGGTACTGAGCTAATAAATCATTATCACTATAATTAAAAACAAAAGTCACT

2081 GAAATCAGGTCAATAGTTACCTTATTAAGTAGTGGGCTAGCTGTGGAATGTTGAAGATCCATTTCCTTTAAAATGATATA

2161 GGTCTTTTCTATCAGTTTGTCTTATATTAAAAAATGCTTTTAAATTTCCTACTATATTAAATACATTCTAATTTGGTCAC

2241 TGATA (SEQ ID NO:17)

TSC21

TSC21 is a novel 171 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

```
  1 actagtcacc aaaatgcttg gttctaagtg gtagagaagg agacacctta gatataatac
 61 aggtcaactt tttgacgtgg ggtgggggtg ggggtggggg tgggggtgaa catcacggtc
121 gcaaataagc agggtttgag ctttgtccag attgtagact taataaaatt y (SEQ ID NO:18)
```

TSC22

TSC22 is a novel 491 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 CAGTTGCAGAAGGGAGAAATCACGGCAGAATCATCGAGAAACCTGAAAAATGAGACCTAGAATGAAGTATTCCAACTCCA

81 AGATTTCCCCGGCAAAGTTCAGCAGCACCGCAGGCGAAGCCCTGGTCCCGCCTTGCAAAATAAGAAGATCCCAACATAAG

161 ACCAAAGAATTCTGCCATGTCTACTGCATGAGACTCCGTTCTGGCCTCACCATAAGAAAGGAGACTAGTTATTTTAGGAA

241 AGAACCCACGAAAAGATATTCACTAAAATCGGGTACCAAGCATGAAGAGAACTTCTCTGCCTATCCACGGGATTCTAGGA

321 AGAGATCCTTGCTTGGCAGTATCCAAGCATTTGCTGCGTCTGTTGACACATTGAGCATCCAAGGAACTTCACTTTTAACA 401

CAGTCTCCTGCCTCCCTGAGTACATACAATGACCAATCTGTTAGTTTTGTTTTGGAGAATGGATGTTATGTGATCAATGT 481

TGACGACTCTG (SEQ ID NO:19)

TSC23

TSC23 is a novel 659 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 ATTTGGAATTTTAAGTTTTATCAATGCTTCTGGAAGCTTAGAACTGTACACGTGTGATGTCAGTCACATAGAGGAATGTG 81

CCCGGACTGCCTCATGCCTTTATTTTCCTTGGTAAATTTGAAGATAGAATGTCTGACTAGCGCAGTGACCAGAAAACAAT 161

GTGGTAGTCAACATCTCAGGCCATATTTTAAGATCCTGTAGAGCACTATTCATTTCAGGTTGCAGATGGAGTATTTTTGA 241

AACATCATTACTATGTAGATGCTTGGATAGGAGTGAGGGGGAGCTAGCAGATTTCCTGTGCCATTTATTCAGCTGATTGA 321

TGTACAGATGTAGGTTTATTTTGTAAAATCCACTGAAAGAATATGGCCACACCCTTGCCTACTTGATAGCATCAATACAG 401

AAGCCAAGAAGGACCACTAAGTAACCCCCTCTTCCCAGGGAGAGCAGCTAGCTTGAAATCTCTCGGATACAATCGATGCG 481

TCTGACCTTTGGGATCCTCACCATATGGGCAAACAATGGGCTTTGCAGGATGAGAGACACCCACTTAAACCTCTGACGAT 561

CTCGAATGGTTCATCTCTTCCGTCATTAACCAGTCATGGAAAACAATCAACAAACTCTGCCACGTGAAATATTTTTTCAG 641

ACTTTTCTAACCCAAGCTT (SEQ ID NO:20)

TSC24

TSC24 is a novel 341 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

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  1 raattcaaac aaagctttgg acaaggcccg gttaaaaagc aaagatgtca agttggcaga
 61 gactcatcag caggaatgct gccagaagtt tgaacagctt tctgaatctg caaaagaaga
121 gctgataaac ttcaaacgga agagagtggc agcatttcga aagaacctaa tcgaaatgtc
181 tgaactggaa ataaagcatg ccagaaacaa cgtctccctg ttgcagagct gcatcgactt
241 attcaagaac aactgacctg tctactctga aggacaccaa tgtgaaagcc agcatcactt
301 gcacttaaat cattactgca aaagaaatag ctttgactag t (SEQ ID NO:21)
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TSC25

TSC25 is a novel 53 bp gene fragment. The nucleic acid was initially identified in a cloned fragment having the following sequence:

1 ggatcctgca aggctttggc cagctcagaa gcggcaaccc ctacacacct agg (SEQ ID NO:22)

GENERAL METHODS

The TSCX nucleic acids and encoded polypeptides can be identified using the information provide above. In some embodiments, the TSCX nucleic acids and polypeptide correspond to nucleic acids or polypeptides which include the various sequences (referenced by SEQ ID NOs) disclosed for each TSCX polypeptide.

In its various aspects and embodiments, the invention includes providing a test cell population which includes at least one cell that is capable of expressing one or more of the sequences TSC 1-142. By "capable of expressing" is meant that the gene is present in an intact form in the cell and can be expressed. Expression of one, some, or all of the TSCX sequences is then detected, if present, and, preferably, measured. Using sequence information provided by the

database entries for the known sequences, or the sequence information for the newly described sequences, expression of the TSCX sequences can be detected (if present) and measured using techniques well known to one of ordinary skill in the art. For example, sequences within the sequence database entries corresponding to TSCX sequences, or within the sequences disclosed herein, can be used to construct probes for detecting TSCX RNA sequences in, *e.g.*, northern blot hybridization analyses or methods which specifically, and, preferably, quantitatively amplify specific nucleic acid sequences. As another example, the sequences can be used to construct primers for specifically amplifying the TSCX sequences in, *e.g.*, amplification-based detection methods such as reverse-transcription based polymerase chain reaction. When alterations in gene expression are associated with gene amplification or deletion, sequence comparisons in test and reference populations can be made by comparing relative amounts of the examined DNA sequences in the test and reference cell populations.

Expression can be also measured at the protein level, *i.e.*, by measuring the levels of polypeptides encoded by the gene products described herein. Such methods are well known in the art and include, *e.g.*, immunoassays based on antibodies to proteins encoded by the genes.

Expression level of the TSCX sequences in the test cell population is then compared to expression levels of the sequences in one or more cells from a reference profile. Expression of sequences in test and control populations of cells can be compared using any art-recognized method for comparing expression of nucleic acid sequences. For example, expression can be compared using GENECALLING® methods as described in US Patent No. 5,871,697 and in Shimkets et al., Nat. Biotechnol. 17:798-803. In various embodiments, the expression of 2, 3, 4, 5, 6, 7,8, 9, 10, 15, 20, 25, 35, 40, 50, 100, 150 or all of the sequences represented by TSC 1-142 are measured. If desired, expression of these sequences can be measured along with other sequences whose expression is known to be altered according to one of the herein described parameters or conditions.

A reference profile is an expression pattern derived from a single reference population or from a plurality of expression patterns. The reference profile can be a database of expression patterns from previously tested cells for which one of the herein-described conditions (*e.g.*, tuberous sclerosis complex associated disorder) is known. Tuberous sclerosis complex assosiated disorders include for example, hamartomas, or hamartias in multiple organ systens, such as the brain, skin, heart or kidney, renal carcinoma, malignamnt angiomyolipoma, hypomelanotic

macules, facila angiofibroma, shagreen patches and ungula fibromas.

In some embodiments, the test cell will be included in a cell sample from a subject known to contain, or to be suspected of having a tuberous sclerosis complex associated disorder. In other embodiments, the cell sample will be derived from a subject from a region known to contain, or suspected of containing, a primary tumor, such as a renal carcinoma. In further embodiments, the cell sample will be derived from a subject from a region known to contain, or suspected of containing, a metastasis of a primary tumor.

Preferably, cells in the reference profile are derived from a tissue type as similar as possible to test cell, *e.g.*, brain, skin, heart or kidney tissue. In some embodiments, the control cell is derived from the same subject as the test cell, *e.g.*, from a region proximal to the region of origin of the test cell.

In some embodiments, the test cell population is compared to multiple reference profiles. Each of the multiple reference profiles may differ in the known parameter or condition. Thus, a test cell population may be compared to a first reference profile known to have an tuberous sclerosis associated disorder, as well as a second reference population known not to have a tuberous sclerosis associated disorder.

In various embodiments, the expression of one or more sequences encoding genes of expressed in distinct gene profiles, as listed in Table 1, is compared. These gene profile include, *e.g.*, "MEF and NSC -/- conserved differential expression" (such as, TSC 1-9), "MEF and NSC -/- opposite differential expression" (TSC 10-18), "NSC Only", (TSC 19-44), and "MEF Only" (TSC 45-57). In some embodiments, expression of members of two or more gene profiles are compared.

Whether or not comparison of the gene expression profile in the test cell population to the reference profile reveals the presence, or degree, of the measured condition depends on the composition of the reference profile. For example, if the profile is composed of cells that have an tuberous sclerosis associated disorder, a similar gene expression level in the test cell population and a reference profile indicates the presence of the tuberous sclerosis associated disorder in the test cell population. Conversely, if the reference profile is composed of cells that do not have an tuberous sclerosis associated disorder, a similar gene expression profile between the test cell population and the reference profile indicates the absence of the tuberous sclerosis associated disorder in the test cell population

In various embodiments, the TSCX sequence in a test cell population is considered comparable in expression level to the expression level of the antileukoprotease sequence if its expression level varies within a factor of 2.0, 1.5, or 1.0 fold to the level of the TSCX transcript in the reference profile. In various embodiments, a TSC sequence in a test cell population can be considered altered in levels of expression if its expression level varies from the reference cell population by more than 1.0, 1.5, 2.0 or more fold from the expression level of the corresponding antileukoprotease sequence in the reference cell population.

If desired, comparison of differentially expressed sequences between a test cell population and a reference profile can be done with respect to a control nucleic acid whose expression is independent of the parameter or condition being measured. Expression levels of the control nucleic acid in the test and reference nucleic acid can be used to normalize signal levels in the compared populations.

The test cell population can be any number of cells, *i.e.*, one or more cells, and can be provided *in vitro, in vivo*, or *ex vivo*.

In other embodiments, the test cell population can be divided into two or more subpopulations. The subpopulations can be created by dividing the first population of cells to create as identical a subpopulation as possible. This will be suitable, in, for example, *in vitro* or *ex vivo* screening methods. In some embodiments, various sub populations can be exposed to a control agent, and/or a test agent, multiple test agents, or, *e.g.*, varying dosages of one or multiple test agents administered together, or in various combinations.

The subject is preferably a mammal. The mammal can be, *e.g.*, a human, non-human primate, mouse, rat, dog, cat, horse, or cow.

DIAGNOSING A TUBEROUS SCLEROSIS COMPLEX ASSOCIATED DISORDER

The invention provides a method of diagnosing or determining the susceptibility of a tuberous sclerosis complex associated disorder, *e.g.*, hamartomas, or hamartias in multiple organ systens, such as the brain, skin, heart or kidney, renal carcinoma, malignant angiomyolipoma, hypomelanotic macules, facila angiofibroma, shagreen patches and ungula fibromas. A tuberous sclerosis complex associated disorder is diagnosed by examining the expression of a nucleic acid encoding a TSCX nucleic acid from a test population of cells from a subject suspected of having a tuberous sclerosis complex associated disorder. The population of cells may contain cells of the

brain, or may alternatively may contain cells the eye, skin, heart, or kidney.

Expression of a TSCX nucleic acid is measured in the test cell and compared to the expression of the sequence in the reference profile. A reference profile can be a TSC disorder positive reference profile. By "TSC disorder positive reference profile" is meant that the reference profile contains cells derived from tissues with a tuberous sclerosis complex associated disorder. Alternatively , the reference profile can be an TSC disorder negative reference profile. By "TSC negative reference profile" is meant that the reference profile contains cells derived from tissues without a tuberous sclerosis complex associated disorder.

When a reference profile is an TSC disorder positive reference profile, a similarity in expression between TSCX sequences in the test population and the reference profile indicates the presence of a tuberous sclerosis complex associated disorder in the subject. Conversely, a difference in expression in the test cell population between TSCX sequences in the test population and the TSC disorder positive reference profile indicates the absence of a tuberous sclerosis complex associated disorder in the subject.

When the reference profile is TSC disorder negative reference profile, an difference in expression pattern between the test cell population and the TSC disorder negative reference profile indicates the presence of a tuberous sclerosis complex associated disorder. Conversely, a similarity in expression expression between TSCX sequences in the test population and the TSC disorder negative reference profile indicates the absence of a tuberous sclerosis complex associated disorder in the subject.

METHODS OF TREATING DISORDERS ASSOCIATED WITH TUBEROUS SCLEROSIS COMPLEX

The invention provides a method for treating tuberous sclerosis complex associated disorders in a subject by administering to a subject in need thereof a compound that modulates the expression of one or more TSCX nucleic acids or polypeptides. Administration can be prophylactic or therapeutic to a subject at risk of (or susceptible to) tuberous sclerosis complex associated disorder. The tuberous sclerosis associated disorder can be, *e.g.*, hamartomas, or hamartias in multiple organ systens, such as the brain, skin, heart or kidney, renal carcinoma, malignant angiomyolipoma, hypomelanotic macules, facila angiofibroma, shagreen patches and ungula fibromas.

The therapeutic method includes decreasing or inhibiting the expression, or function, or

TSCX nucleic acids in the diseased cell relative to normal cells of the tissue type from which the diseased cells are derived. In these methods, the subject is treated with an effective amount of a compound, which decreases the amount of a TSCX nucleic acid or polypeptide in the subject. Administration can be systemic or local, *e.g.*, in the immediate vicinity of, the subject's diseased cells. Expression can be inhibited in any of several ways known in the art. For example, expression can be inhibited by administering to the subject a nucleic acid that inhibits, or antagonizes, the expression of the TSCX. In one embodiment, an antisense oligonucleotide can be administered which disrupts expression of a TSCX nucleic acid.

Alternatively, the function a TSCX can be inhibited by administering a compound that binds to or otherwise inhibits the function of the TSCX gene products. The compound can be, *e.g.*, an antibody to a polypeptide encoded by a TSCX nucleic acid..

These modulatory methods can be performed *ex vivo* or *in vitro* (*e.g.*, by culturing the cell with the agent) or, alternatively, *in vivo* (*e.g.*, by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity TSCX proteins or nucleic acid molecules. In one embodiment, the method involves administering an agent (*e.g.*, an agent identified by a screening assay described herein), or combination of agents that modulates (*e.g.*, upregulates or downregulates) expression or activity of TSCX nucleic acids or polypeptides. In another embodiment, the method involves administering a protein or combination of proteins or a nucleic acid molecule or combination of nucleic acid, molecules as therapy to compensate for aberrant expression or activity of a TSCX nucleic acid.

Therapeutics that may be utilized include, *e.g.*, (*i*) a polypeptide, or analogs, derivatives, fragments or homologs thereof of the overexpressed sequence; (*ii*) antibodies to the overexpressed sequence; (*iii*) antisense nucleic acids or nucleic acids that are "dysfunctional" (*i.e.*, due to a heterologous insertion within the coding sequences of coding sequences of one or more overexpressed or underexpressed sequences); or (*v*) modulators (*i.e.*, inhibitors, agonists and antagonists that alter the interaction between an overexpressed polypeptide and its binding partner. The dysfunctional antisense molecules are utilized to "knockout" endogenous function of a polypeptide by homologous recombination (see, *e.g.*, Capecchi, *Science* 244: 1288-1292 1989) Increased or decreased levels can be readily detected by quantifying peptide and/or RNA, by obtaining a patient tissue sample (*e.g.*, from biopsy tissue) and assaying it *in vitro* for RNA or

peptide levels, structure and/or activity of the expressed peptides (or mRNAs of a gene whose expression is altered). Methods that are well-known within the art include, but are not limited to, immunoassays (*e.g.*, by Western blot analysis, immunoprecipitation followed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis, immunocytochemistry, etc.) and/or hybridization assays to detect expression of mRNAs (*e.g.*, Northern assays, dot blots, *in situ* hybridization, etc.).

Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of aberrant gene expression, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of aberrant expression detected, the agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

SCREENING ASSAYS FOR IDENTIFYING A CANDIDATE THERAPEUTIC AGENT FOR TREATING OR PREVENTING TUBEROUS SCLEROSIS ASSOCIATED DISORDER

The differentially expressed sequences disclosed herein can also be used to identify candidate therapeutic agents to treat or prevent tuberous sclerosis associated disorders. The therapeutic agent can be identified by providing a cell population that includes cells capable of expressing TSCX nucelic acids. Expression of the nucleic acid sequences in the test cell population is then compared to the expression of the nucleic acid sequences in a reference cell population, which is a cell population that has not been exposed to the test agent, or, in some embodiments, a cell population exposed the test agent. Comparison can be performed on test and reference samples measured concurrently or at temporally distinct times. An example of the latter is the use of compiled expression information, *e.g.*, a sequence database, which assembles information about expression levels of known sequences following administration of various agents. For example, alteration of expression levels following administration of test agent can be compared to the expression changes observed in the nucleic acid sequences following administration of a control agent.

An decrease in expression of the nucleic acid sequence in the test cell population compared to the expression of the nucleic acid sequence in the reference cell population that has not been exposed to the test agent indicates the test agent is a canidate therapuetic agent.

The test agent can be a compound not previously described or can be a previously known compound but which is not known to be an agent for treating tuberous sclerosis complex

disorders.

The invention also includes a compound identified according to this screening method.

An agent effective in stimulating expression of underexpressed genes, or in suppressing expression of overexpressed genes can be further tested for its ability to prevent the tuberous sclerosis complex associated disorders, and as a potential therapeutic useful for the treatment of such pathophysiology. Further evaluation of the clinical usefulness of such a compound can be performed using standard methods of evaluating toxicity and clinical effectiveness.

SELECTING A THERAPEUTIC AGENT FOR TREATING TUBEROUS SCLEROSIS COMPLEX ASSOCIATED DISORDER THAT IS APPROPRIATE FOR A PARTICULAR INDIVIDUAL

Differences in the genetic makeup of individuals can result in differences in their relative abilities to metabolize various drugs. An agent that is metabolized in a subject to act as a therapeutic agent can manifest itself by inducing a change in gene expression pattern from that characteristic of a pathophysiologic state to a gene expression pattern characteristic of a non-pathophysiologic state. Accordingly, the differentially expressed TSCX sequences disclosed herein allow for a putative therapeutic or prophylactic agent to be tested in a test cell population from a selected subject in order to determine if the agent is a suitable therapeutic agent in the subject.

To identify a therapeutic agent, that is appropriate for a specific subject, a test cell population from the subject is exposed to a therapeutic agent, and the expression of one or more of TSCX 1-141.

In some embodiments, the agent is first mixed with a cell extract, which contains enzymes that metabolize drugs into an active form. The activated form of the therapeutic agent can then be mixed with the test cell population and gene expression measured. Preferably, the cell population is contacted *ex vivo* with the agent or activated form of the agent.

Expression of the nucleic acid sequences in the test cell population is then compared to the expression of the nucleic acid sequences a reference cell population. The reference cell population includes at least one cell whose tuberous sclerosis complex status is known. By

"tuberous sclerosis complex status is meant, whether or not the reference cell population contains cells known to have tuberous sclerosis complex subject.

The test agent can be any compound or composition.

ASSESSING EFFICACY OF TREATMENT OF A TUBEROUS SCLEROSIS COMPLEX ASSOCIATED DISORDER IN A SUBJECT

The differentially expressed TSCX sequences identified herein also allow for the course of treatment of a tuberous sclerosis complex associated disorder to be monitored. In this method, a test cell population is provided from a subject undergoing treatment for a tuberous sclerosis complex associated disorder. If desired, test cell populations can be taken from the subject at various time points before, during, or after treatment. Expression of one or more of the TSCX sequences, *e.g.*, TSCXs: 1-142, in the cell population is then measured and compared to a reference cell population which includes cells whose pathophysiologic state is known. Preferably, the reference cells not been exposed to the treatment.

If the reference cell population contains no cells exposed to the treatment, a similarity in expression between TSCX sequences in the test cell population and the reference cell population indicates that the treatment is efficacious. However, a difference in expression between TSCX sequences in the test population and this reference cell population indicates the treatment is not efficacious.

By "efficacious" is meant that the treatment leads to a decrease in the pathophysiology in a subject. When treatment is applied prophylactically, "efficacious" means that the treatment retards or prevents a pathophysiology. .

Efficaciousness can be determined in association with any known method for treating the particular pathophysiology

ASSESSING THE PROGNOSIS OF A SUBJECT WITH A TUBEROUS SCLEROSIS COMPLEX ASSOCIATED DISORDER

Also provided is a method of assessing the prognosis of a subject with a tuberous sclerosis compelx associated disorder by comparing the expression of a TSCX nucleic acid in a test cell

population to the expression of the sequences in a reference profile derived from patients over a spectrum of disease stages. By comparing gene expression of a TSCX nucleic acid in the test cell population and the reference profile, or by comparing the pattern of gene expression overtime in test cell populations derived from the subject, the prognosis of the subject can be assessed.

The reference profile includes primarily noncancerous or cancerous cells. A reference profile which includes primarily noncancerous cells is a non-cancer reference profile. A reference profile which includes primarily cancerous cells is a cancer reference profile. In some embodiments the cancer reference profile includes primarily disseminated cancerous cells. When the reference profile includes primarily noncancerous cells, an increase of expression of TSCX nucleic acids in the test cell population, indicates less favorable prognosis. Conversely, when the reference profile includes primarily cancerous cells, an decrease of expression of TSCX nucleic acids in the test cell population, indicates more favorable prognosis.

PHARMACEUTICAL COMPOSITIONS

In another aspect the invention includes pharmaceutical, or therapeutic, compositions containing one or more therapeutic compounds described herein. Pharmaceutical formulations may include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All such pharmacy methods include the steps of bringing into association the active compound with liquid carriers or finely divided solid carriers or both as needed and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units, such as capsules, cachets or tablets, each containing a predetermined amount of the active ingredient; as a powder or granules; or as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus electuary or paste, and be in a pure form, i.e., without a carrier. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrant or wetting agents. A tablet may be made by compression or molding, optionally with one or more formulational ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active

ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may be coated according to methods well known in the art. Oral fluid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives. The tablets may optionally be formulated so as to provide slow or controlled release of the active ingredient therein.

Formulations for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline, water-for-injection, immediately prior to use. Alternatively, the formulations may be presented for continuous infusion. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Formulations for rectal administration may be presented as a suppository with the usual carriers such as cocoa butter or polyethylene glycol. Formulations for topical administration in the mouth, for example buccally or sublingually, include lozenges, comprising the active ingredient in a flavored base such as sucrose and acacia or tragacanth, and pastilles comprising the active ingredient in a base such as gelatin and glycerin or sucrose and acacia. For intra-nasal administration the compounds of the invention may be used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs.

For administration by inhalation the compounds are conveniently delivered from an insufflator, nebulizer, pressurized packs or other convenient means of delivering an aerosol spray. Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichiorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

Alternatively, for administration by inhalation or insufflation, the compounds may take the form of a dry powder composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form, in for example, capsules, cartridges, gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insuffiator.

When desired, the above described formulations, adapted to give sustained release of the active ingredient, may be employed. The pharmaceutical compositions may also contain other active ingredients such as antimicrobial agents, immunosuppressants or preservatives.

It should be understood that in addition to the ingredients particularly mentioned above, the formulations of this invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for oral administration may include flavoring agents.

Preferred unit dosage formulations are those containing an effective dose, as recited below, or an appropriate fraction thereof, of the active ingredient.

For each of the aforementioned conditions, the compositions may be administered orally or via injection at a dose of from about 0.1 to about 250 mg/kg per day. The dose range for adult humans is generally from about 5 mg to about 17.5 g/day, preferably about 5 mg to about 10 g/day, and most preferably about 100 mg to about 3 g/day. Tablets or other unit dosage forms of presentation provided in discrete units may conveniently contain an amount which is effective at such dosage or as a multiple of the same, for instance, units containing about 5 mg to about 500 mg, usually from about 100 mg to about 500 mg.

The pharmaceutical composition preferably is administered orally or by injection (intravenous or subcutaneous), and the precise amount administered to a subject will be the responsibility of the attendant physician. However, the dose employed will depend upon a number of factors, including the age and sex of the subject, the precise disorder being treated, and its severity. Also the route of administration may vary depending upon the condition and its severity.

TSCX NUCLEIC ACIDS

Also provided in the invention are novel nucleic acid comprising a nucleic acid sequence selected from the group consisting of TSC: 1-8, 10-12, and 15-25 (SEQ ID NO: 1-22) or its complement, as well as vectors and cells including these nucleic acids.

Thus, one aspect of the invention pertains to isolated TSCX nucleic acid molecules that encode TSCX proteins or biologically active portions thereof. Also included are nucleic acid fragments sufficient for use as hybridization probes to identify TSCX-encoding nucleic acids (*e.g.*, TSCX mRNA) and fragments for use as polymerase chain reaction (PCR) primers for the amplification or mutation of TSCX nucleic acid molecules. As used herein, the term "nucleic acid molecule" is intended to include DNA molecules (*e.g.*, cDNA or genomic DNA), RNA molecules (*e.g.*, mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA.

"Probes" refer to nucleic acid sequences of variable length, preferably between at least about 10 nucleotides (nt) or as many as about, *e.g.*, 6,000 nt, depending on use. Probes are used in the detection of identical, similar, or complementary nucleic acid sequences. Longer length probes are usually obtained from a natural or recombinant source, are highly specific and much slower to hybridize than oligomers. Probes may be single- or double-stranded and designed to have specificity in PCR, membrane-based hybridization technologies, or ELISA-like technologies.

An "isolated" nucleic acid molecule is one that is separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. Examples of isolated nucleic acid molecules include, but are not limited to, recombinant DNA molecules contained in a vector, recombinant DNA molecules maintained in a heterologous host cell, partially or

substantially purified nucleic acid molecules, and synthetic DNA or RNA molecules. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated TSCX nucleic acid molecule can contain less than about 50 kb, 25 kb, 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material or culture medium when produced by recombinant techniques, or of chemical precursors or other chemicals when chemically synthesized.

A nucleic acid molecule of the present invention, *e.g.*, a nucleic acid molecule having the nucleotide sequence of any of TSC: 1-8, 10-12, and 15-25, or a complement of any of these nucleotide sequences, can be isolated using standard molecular biology techniques and the sequence information provided herein. Using all or a portion of these nucleic acid sequences as a hybridization probe, TSCX nucleic acid sequences can be isolated using standard hybridization and cloning techniques (*e.g.*, as described in Sambrook *et al.*, eds., MOLECULAR CLONING: A LABORATORY MANUAL 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; and Ausubel, *et al.*, eds., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993.)

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to *TSCX* nucleotide sequences can be prepared by standard synthetic techniques, *e.g.*, using an automated DNA synthesizer.

As used herein, the term "oligonucleotide" refers to a series of linked nucleotide residues, which oligonucleotide has a sufficient number of nucleotide bases to be used in a PCR reaction. A short oligonucleotide sequence may be based on, or designed from, a genomic or cDNA sequence and is used to amplify, confirm, or reveal the presence of an identical, similar or complementary DNA or RNA in a particular cell or tissue. Oligonucleotides comprise portions of

a nucleic acid sequence having at least about 10 nt and as many as 50 nt, preferably about 15 nt to 30 nt. They may be chemically synthesized and may be used as probes.

In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in TSCX: :1-7, 10-13, 19-34, 45-53, 58-85, 111-113, 120, 130, 132-134 and 138 . In another embodiment, an isolated nucleic acid molecule of the invention comprises a nucleic acid molecule that is a complement of the nucleotide sequence shown in any of these sequences, or a portion of any of these nucleotide sequences. A nucleic acid molecule that is complementary to the nucleotide sequence shown in TSC: 1-8, 10-12, and 15-25 is one that is sufficiently complementary to the nucleotide sequence shown, such that it can hydrogen bond with little or no mismatches to the nucleotide sequences shown, thereby forming a stable duplex.

As used herein, the term "complementary" refers to Watson-Crick or Hoogsteen base pairing between nucleotides units of a nucleic acid molecule, and the term "binding" means the physical or chemical interaction between two polypeptides or compounds or associated polypeptides or compounds or combinations thereof. Binding includes ionic, non-ionic, Von der Waals, hydrophobic interactions, etc. A physical interaction can be either direct or indirect. Indirect interactions may be through or due to the effects of another polypeptide or compound. Direct binding refers to interactions that do not take place through, or due to, the effect of another polypeptide or compound, but instead are without other substantial chemical intermediates.

Moreover, the nucleic acid molecule of the invention can comprise only a portion of the nucleic acid sequence of TSC: 1-8, 10-12, and 15-25 *e.g.*, a fragment that can be used as a probe or primer or a fragment encoding a biologically active portion of TSCX. Fragments provided herein are defined as sequences of at least 6 (contiguous) nucleic acids or at least 4 (contiguous) amino acids, a length sufficient to allow for specific hybridization in the case of nucleic acids or for specific recognition of an epitope in the case of amino acids, respectively, and are at most some portion less than a full length sequence. Fragments may be derived from any contiguous portion of a nucleic acid or amino acid sequence of choice. Derivatives are nucleic acid sequences or amino acid sequences formed from the native compounds either directly or by modification or partial substitution. Analogs are nucleic acid sequences or amino acid sequences that have a structure similar to, but not identical to, the native compound but differs from it in respect to

certain components or side chains. Analogs may be synthetic or from a different evolutionary origin and may have a similar or opposite metabolic activity compared to wild type.

Derivatives and analogs may be full length or other than full length, if the derivative or analog contains a modified nucleic acid or amino acid, as described below. Derivatives or analogs of the nucleic acids or proteins of the invention include, but are not limited to, molecules comprising regions that are substantially homologous to the nucleic acids or proteins of the invention, in various embodiments, by at least about 45%, 50%, 70%, 80%, 95%, 98%, or even 99% identity (with a preferred identity of 80-99%) over a nucleic acid or amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art, or whose encoding nucleic acid is capable of hybridizing to the complement of a sequence encoding the aforementioned proteins under stringent, moderately stringent, or low stringent conditions. See *e.g.* Ausubel, *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, NY, 1993, and below. An exemplary program is the Gap program (Wisconsin Sequence Analysis Package, Version 8 for UNIX, Genetics Computer Group, University Research Park, Madison, WI) using the default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2: 482-489, which in incorporated herein by reference in its entirety).

A "homologous nucleic acid sequence" or "homologous amino acid sequence," or variations thereof, refer to sequences characterized by a homology at the nucleotide level or amino acid level as discussed above. Homologous nucleotide sequences encode those sequences coding for isoforms of a TSCX polypeptide. Isoforms can be expressed in different tissues of the same organism as a result of, for example, alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the present invention, homologous nucleotide sequences include nucleotide sequences encoding for a TSCX polypeptide of species other than humans, including, but not limited to, mammals, and thus can include, *e.g.*, mouse, rat, rabbit, dog, cat cow, horse, and other organisms. Homologous nucleotide sequences also include, but are not limited to, naturally occurring allelic variations and mutations of the nucleotide sequences set forth herein. A homologous nucleotide sequence does not, however, include the nucleotide sequence encoding a human TSCX protein. Homologous nucleic acid sequences include those nucleic acid sequences that encode conservative amino acid substitutions (see below) in a TSCX polypeptide, as well as a

polypeptide having a TSCX activity. A homologous amino acid sequence does not encode the amino acid sequence of a human TSCX polypeptide.

The nucleotide sequence determined from the cloning of human TSCX genes allows for the generation of probes and primers designed for use in identifying and/or cloning TSCX homologues in other cell types, *e.g.*, from other tissues, as well as TSCX homologues from other mammals. The probe/primer typically comprises a substantially purified oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 25, 50, 100, 150, 200, 250, 300, 350 or 400 consecutive sense strand nucleotide sequence of a nucleic acid comprising a TSCX sequence, or an anti-sense strand nucleotide sequence of a nucleic acid comprising a TSCX sequence, or of a naturally occurring mutant of these sequences.

Probes based on human TSCX nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or homologous proteins. In various embodiments, the probe further comprises a label group attached thereto, *e.g.*, the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a diagnostic test kit for identifying cells or tissue which misexpress a TSCX protein, such as by measuring a level of a TSCX-encoding nucleic acid in a sample of cells from a subject *e.g.*, detecting TSCX mRNA levels or determining whether a genomic TSCX gene has been mutated or deleted.

"A polypeptide having a biologically active portion of TSCX" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. A nucleic acid fragment encoding a "biologically active portion of TSCX" can be prepared by isolating a portion of TSC: 1-8, 10-12, and 15-25, that encodes a polypeptide having a TSCX biological activity, expressing the encoded portion of TSCX protein (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of TSCX. For example, a nucleic acid fragment encoding a biologically active portion of a TSCX polypeptide can optionally include an ATP-binding domain. In another embodiment, a nucleic acid fragment encoding a biologically active portion of TSCX includes one or more regions.

TSCX VARIANTS

The invention further encompasses nucleic acid molecules that differ from the disclosed or referenced TSCX nucleotide sequences due to degeneracy of the genetic code. These nucleic acids thus encode the same TSCX protein as that encoded by nucleotide sequence comprising a TSCX nucleic acid as shown in, *e.g.*, TSC: 1-8, 10-12, and 15-25

In addition to the rat TSCX nucleotide sequence shown in TSC: 1-8, 10-12, and 15-25, it will be appreciated by those skilled in the art that DNA sequence polymorphisms that lead to changes in the amino acid sequences of a TSCX polypeptide may exist within a population (*e.g.*, the human population). Such genetic polymorphism in the TSCX gene may exist among individuals within a population due to natural allelic variation. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules comprising an open reading frame encoding a TSCX protein, preferably a mammalian TSCX protein. Such natural allelic variations can typically result in 1-5% variance in the nucleotide sequence of the TSCX gene. Any and all such nucleotide variations and resulting amino acid polymorphisms in TSCX that are the result of natural allelic variation and that do not alter the functional activity of TSCX are intended to be within the scope of the invention.

Moreover, nucleic acid molecules encoding TSCX proteins from other species, and thus that have a nucleotide sequence that differs from the human sequence of TSC: 1-8, 10-12, and 15-25, are intended to be within the scope of the invention. Nucleic acid molecules corresponding to natural allelic variants and homologues of the TSCX DNAs of the invention can be isolated based on their homology to the human TSCX nucleic acids disclosed herein using the human cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques under stringent hybridization conditions. For example, a soluble human TSCX DNA can be isolated based on its homology to human membrane-bound TSCX. Likewise, a membrane-bound human TSCX DNA can be isolated based on its homology to soluble human TSCX.

Accordingly, in another embodiment, an isolated nucleic acid molecule of the invention is at least 6 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising the nucleotide sequence of TSC: 1-8, 10-12, and 15-25. In another embodiment, the nucleic acid is at least 10, 25, 50, 100, 250 or 500 nucleotides in length. In

another embodiment, an isolated nucleic acid molecule of the invention hybridizes to the coding region. As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% homologous to each other typically remain hybridized to each other.

Homologs (*i.e.*, nucleic acids encoding TSCX proteins derived from species other than human) or other related sequences (*e.g.*, paralogs) can be obtained by low, moderate or high stringency hybridization with all or a portion of the particular human sequence as a probe using methods well known in the art for nucleic acid hybridization and cloning.

As used herein, the phrase "stringent hybridization conditions" refers to conditions under which a probe, primer or oligonucleotide will hybridize to its target sequence, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures than shorter sequences. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength, pH and nucleic acid concentration) at which 50% of the probes complementary to the target sequence hybridize to the target sequence at equilibrium. Since the target sequences are generally present at excess, at Tm, 50% of the probes are occupied at equilibrium. Typically, stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes, primers or oligonucleotides (e.g., 10 nt to 50 nt) and at least about 60°C for longer probes, primers and oligonucleotides. Stringent conditions may also be achieved with the addition of destabilizing agents, such as formamide.

Stringent conditions are known to those skilled in the art and can be found in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Preferably, the conditions are such that sequences at least about 65%, 70%, 75%, 85%, 90%, 95%, 98%, or 99% homologous to each other typically remain hybridized to each other. A non-limiting example of stringent hybridization conditions is hybridization in a high salt buffer comprising 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA, and 500 mg/ml denatured salmon sperm DNA at 65°C. This hybridization is followed by one or more washes in 0.2X SSC, 0.01% BSA at 50°C. An isolated nucleic acid molecule of the invention that

hybridizes under stringent conditions to the sequence of TSC: 1-8, 10-12, and 15-25 corresponds to a naturally occurring nucleic acid molecule. As used herein, a "naturally-occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (*e.g.*, encodes a natural protein).

In a second embodiment, a nucleic acid sequence that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of TSC: 1-8, 10-12, and 15-25 or fragments, analogs or derivatives thereof, under conditions of moderate stringency is provided. A non-limiting example of moderate stringency hybridization conditions are hybridization in 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 mg/ml denatured salmon sperm DNA at 55°C, followed by one or more washes in 1X SSC, 0.1% SDS at 37°C. Other conditions of moderate stringency that may be used are well known in the art. See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY.

In a third embodiment, a nucleic acid that is hybridizable to the nucleic acid molecule comprising the nucleotide sequence of TSC: 1-8, 10-12, and 15-25or fragments, analogs or derivatives thereof, under conditions of low stringency, is provided. A non-limiting example of low stringency hybridization conditions are hybridization in 35% formamide, 5X SSC, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 mg/ml denatured salmon sperm DNA, 10% (wt/vol) dextran sulfate at 40°C, followed by one or more washes in 2X SSC, 25 mM Tris-HCl (pH 7.4), 5 mM EDTA, and 0.1% SDS at 50°C. Other conditions of low stringency that may be used are well known in the art (*e.g.*, as employed for cross-species hybridizations). See, *e.g.*, Ausubel *et al.* (eds.), 1993, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY, and Kriegler, 1990, GENE TRANSFER AND EXPRESSION, A LABORATORY MANUAL, Stockton Press, NY; Shilo et al., 1981, *Proc Natl Acad Sci USA 78*: 6789-6792.

CONSERVATIVE MUTATIONS

In addition to naturally-occurring allelic variants of the TSCX sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced into an TSCX nucleic acid or directly into an TSCX polypeptide sequence without altering the functional

ability of the TSCX protein. In some embodiments, the nucleotide sequence of TSC: 1-8, 10-12, and 15-25will be altered, thereby leading to changes in the amino acid sequence of the encoded TSCX protein. For example, nucleotide substitutions that result in amino acid substitutions at various "non-essential" amino acid residues can be made in the sequence of TSC: 1-8, 10-12, and 15-25. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of TSCX without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. For example, amino acid residues that are conserved among the TSCX proteins of the present invention, are predicted to be particularly unamenable to alteration.

In addition, amino acid residues that are conserved among family members of the TSCX proteins of the present invention, are also predicted to be particularly unamenable to alteration. As such, these conserved domains are not likely to be amenable to mutation. Other amino acid residues, however, (*e.g.*, those that are not conserved or only semi-conserved among members of the TSCX proteins) may not be essential for activity and thus are likely to be amenable to alteration.

Another aspect of the invention pertains to nucleic acid molecules encoding TSCX proteins that contain changes in amino acid residues that are not essential for activity. Such TSCX proteins differ in amino acid sequence from the amino acid sequences of polypeptides encoded by nucleic acids containing TSC: 1-8, 10-12, and 15-25, yet retain biological activity. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises an amino acid sequence at least about 45% homologous, more preferably 60%, and still more preferably at least about 70%, 80%, 90%, 95%, 98%, and most preferably at least about 99% homologous to the amino acid sequence of the amino acid sequences of polypeptides encoded by nucleic acids comprising TSC: 1-8, 10-12, and 15-25.

An isolated nucleic acid molecule encoding a TSCX protein homologous to can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of a nucleic acid comprising TSC: 1-8, 10-12, and 15-25, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein.

Mutations can be introduced into a nucleic acid comprising TSC: 1-8, 10-12, and 15-25 by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a predicted nonessential amino acid residue in TSCX is replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a TSCX coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for TSCX biological activity to identify mutants that retain activity. Following mutagenesis of the nucleic acid, the encoded protein can be expressed by any recombinant technology known in the art and the activity of the protein can be determined.

In one embodiment, a mutant TSCX protein can be assayed for (1) the ability to form protein:protein interactions with other TSCX proteins, other cell-surface proteins, or biologically active portions thereof, (2) complex formation between a mutant TSCX protein and a TSCX ligand; (3) the ability of a mutant TSCX protein to bind to an intracellular target protein or biologically active portion thereof; (*e.g.*, avidin proteins); (4) the ability to bind ATP; or (5) the ability to specifically bind a TSCX protein antibody.

In other specific embodiments, the nucleic acid is RNA or DNA. The fragment or the fragment of the complementary polynucleotide sequence is between about 10 and about 100 nucleotides in length, *e.g.*, between about 10 and about 90 nucleotides in length, or about 10 and about 75 nucleotides in length, about 10 and about 50 bases in length, about 10 and about 40 bases in length, or about 15 and about 30 bases in length.

ANTISENSE

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of a TSCX sequence or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire TSCX coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a TSCX protein, or antisense nucleic acids complementary to a nucleic acid comprising a TSCX nucleic acid sequence are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding TSCX. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding TSCX. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding TSCX disclosed herein, antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of TSCX mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of TSCX mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of TSCX mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to

increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a TSCX protein to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic

acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA -DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

RIBOZYMES AND PNA MOIETIES

In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave TSCX mRNA transcripts to thereby inhibit translation of TSCX mRNA. A ribozyme having specificity for a TSCX-encoding nucleic acid can be designed based upon the nucleotide sequence of a TSCX DNA disclosed herein. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a TSCX-encoding mRNA. See, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742. Alternatively, TSCX mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, TSCX gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a TSCX nucleic acid (*e.g.*, the TSCX promoter and/or enhancers) to form triple helical structures that prevent transcription of the TSCX gene in target

cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of TSCX can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of TSCX can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of TSCX can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of TSCX can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras of TSCX can be generated that may combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For

example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, Proc. *Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents. (See, *e.g.*, Zon, 1988, *Pharm. Res.* 5: 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

TSCX POLYPEPTIDES

One aspect of the invention pertains to isolated TSCX proteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-TSCX antibodies. In one embodiment, native TSCX proteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques. In another embodiment, TSCX proteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a TSCX protein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which

the TSCX protein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of TSCX protein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of TSCX protein having less than about 30% (by dry weight) of non-TSCX protein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-TSCX protein, still more preferably less than about 10% of non-TSCX protein, and most preferably less than about 5% non-TSCX protein. When the TSCX protein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of TSCX protein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of TSCX protein having less than about 30% (by dry weight) of chemical precursors or non-*TSCX* chemicals, more preferably less than about 20% chemical precursors or non-TSCX chemicals, still more preferably less than about 10% chemical precursors or non-TSCX chemicals, and most preferably less than about 5% chemical precursors or non-TSCX chemicals.

Biologically active portions of a TSCX protein include peptides comprising amino acid sequences sufficiently homologous to or derived from the amino acid sequence of the TSCX protein, *e.g.*, the amino acid sequence encoded by a nucleic acid comprising TSCX 1-20 that include fewer amino acids than the full length TSCX proteins, and exhibit at least one activity of a TSCX protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the TSCX protein. A biologically active portion of a TSCX protein can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length.

A biologically active portion of a TSCX protein of the present invention may contain at least one of the above-identified domains conserved between the TSCX proteins. An alternative biologically active portion of a TSCX protein may contain at least two of the above-identified

domains. Another biologically active portion of a TSCX protein may contain at least three of the above-identified domains. Yet another biologically active portion of a TSCX protein of the present invention may contain at least four of the above-identified domains.

Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the functional activities of a native TSCX protein.

In some embodiments, the TSCX protein is substantially homologous to one of these TSCX proteins and retains its the functional activity, yet differs in amino acid sequence due to natural allelic variation or mutagenesis, as described in detail below.

In specific embodiments, the invention includes an isolated polypeptide comprising an amino acid sequence that is 80% or more identical to the sequence of a polypeptide whose expression is modulated in a mammal to which TSCXic agent is administered.

DETERMINING HOMOLOGY BETWEEN TWO OR MORE SEQUENCES

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (*i.e.*, as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity").

The nucleic acid sequence homology may be determined as the degree of identity between two sequences. The homology may be determined using computer programs known in the art, such as GAP software provided in the GCG program package. See *Needleman and Wunsch* 1970 *J Mol Biol* 48: 443-453. Using GCG GAP software with the following settings for nucleic acid sequence comparison: GAP creation penalty of 5.0 and GAP extension penalty of 0.3, the coding region of the analogous nucleic acid sequences referred to above exhibits a degree of identity preferably of at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%, with the CDS (encoding)

part of a DNA sequence comprising TSCX: :1-7, 10-13, 19-34, 45-53, 58-85, 111-113, 120, 130, 132-134 and 138.

The term "sequence identity" refers to the degree to which two polynucleotide or polypeptide sequences are identical on a residue-by-residue basis over a particular region of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over that region of comparison, determining the number of positions at which the identical nucleic acid base (*e.g.,* A, T, C, G, U, or I, in the case of nucleic acids) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the region of comparison *(i.e.,* the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The term "substantial identity" as used herein denotes a characteristic of a polynucleotide sequence, wherein the polynucleotide comprises a sequence that has at least 80 percent sequence identity, preferably at least 85 percent identity and often 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison region.

CHIMERIC AND FUSION PROTEINS

The invention also provides TSCX chimeric or fusion proteins. As used herein, an TSCX "chimeric protein" or "fusion protein" comprises an TSCX polypeptide operatively linked to a non-TSCX polypeptide. A "TSCX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to TSCX, whereas a "non-TSCX polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein that is not substantially homologous to the TSCX protein, *e.g.*, a protein that is different from the TSCX protein and that is derived from the same or a different organism. Within an TSCX fusion protein the TSCX polypeptide can correspond to all or a portion of an TSCX protein. In one embodiment, an TSCX fusion protein comprises at least one biologically active portion of an TSCX protein. In another embodiment, an TSCX fusion protein comprises at least two biologically active portions of an TSCX protein. In yet another embodiment, an TSCX fusion protein comprises at least three biologically active portions of an TSCX protein. Within the fusion protein, the term "operatively linked" is intended to indicate that the TSCX polypeptide and the non-TSCX polypeptide are fused in-frame to each other. The non-TSCX polypeptide can be fused to the N-terminus or C-terminus of the TSCX polypeptide.

For example, in one embodiment an TSCX fusion protein comprises an TSCX domain operably linked to the extracellular domain of a second protein. Such fusion proteins can be further utilized in screening assays for compounds which modulate TSCX activity (such assays are described in detail below).

In yet another embodiment, the fusion protein is a GST-TSCX fusion protein in which the TSCX sequences are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences. Such fusion proteins can facilitate the purification of recombinant TSCX.

In another embodiment, the fusion protein is an TSCX protein containing a heterologous signal sequence at its N-terminus. For example, a native TSCX signal sequence can be removed and replaced with a signal sequence from another protein. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of TSCX can be increased through use of a heterologous signal sequence.

In yet another embodiment, the fusion protein is an TSCX-immunoglobulin fusion protein in which the TSCX sequences comprising one or more domains are fused to sequences derived from a member of the immunoglobulin protein family. The TSCX-immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a TSCX ligand and a TSCX protein on the surface of a cell, to thereby suppress TSCX-mediated signal transduction *in vivo*. The TSCX-immunoglobulin fusion proteins can be used to affect the bioavailability of an TSCX cognate ligand. Inhibition of the TSCX ligand/TSCX interaction may be useful therapeutically for both the treatments of proliferative and differentiative disorders, as well as modulating (*e.g.* promoting or inhibiting) cell survival. Moreover, the TSCX-immunoglobulin fusion proteins of the invention can be used as immunogens to produce anti-TSCX antibodies in a subject, to purify TSCX ligands, and in screening assays to identify molecules that inhibit the interaction of TSCX with a TSCX ligand.

An TSCX chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to

provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, Ausubel *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). An TSCX-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the TSCX protein.

TSCX AGONISTS AND ANTAGONISTS

The present invention also pertains to variants of the TSCX proteins that function as either TSCX agonists (mimetics) or as TSCX antagonists. Variants of the TSCX protein can be generated by mutagenesis, *e.g.*, discrete point mutation or truncation of the TSCX protein. An agonist of the TSCX protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the TSCX protein. An antagonist of the TSCX protein can inhibit one or more of the activities of the naturally occurring form of the TSCX protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the TSCX protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the TSCX proteins.

Variants of the TSCX protein that function as either TSCX agonists (mimetics) or as TSCX antagonists can be identified by screening combinatorial libraries of mutants, *e.g.*, truncation mutants, of the TSCX protein for TSCX protein agonist or antagonist activity. In one embodiment, a variegated library of TSCX variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of TSCX variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential TSCX sequences is

expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display) containing the set of TSCX sequences therein. There are a variety of methods which can be used to produce libraries of potential TSCX variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential TSCX sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, *e.g.*, Narang (1983) *Tetrahedron* 39:3; Itakura *et al.* (1984) *Annu Rev Biochem* 53:323; Itakura *et al.* (1984) *Science* 198:1056; Ike *et al.* (1983) *Nucl Acid Res* 11:477.

POLYPEPTIDE LIBRARIES

In addition, libraries of fragments of the TSCX protein coding sequence can be used to generate a variegated population of TSCX fragments for screening and subsequent selection of variants of an TSCX protein. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a TSCX coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA that can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the TSCX protein.

Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of TSCX proteins. The most widely used techniques, which are amenable to high throughput analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new

technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify TSCX variants (Arkin and Yourvan (1992) PNAS 89:7811-7815; Delgrave *et al.* (1993) Protein Engineering 6:327-331).

ANTI-TSCX ANTIBODIES

An isolated TSCX protein, or a portion or fragment thereof, can be used as an immunogen to generate antibodies that bind TSCX using standard techniques for polyclonal and monoclonal antibody preparation. The full-length TSCX protein can be used or, alternatively, the invention provides antigenic peptide fragments of TSCX for use as immunogens. The antigenic peptide of TSCX comprises at least 8 amino acid residues of the amino acid sequence encoded by a nucleic acid comprising the nucleic acid sequence shown in TSC: 1-8, 10-12, and 15-25 and encompasses an epitope of TSCX such that an antibody raised against the peptide forms a specific immune complex with TSCX. Preferably, the antigenic peptide comprises at least 10 amino acid residues, more preferably at least 15 amino acid residues, even more preferably at least 20 amino acid residues, and most preferably at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of TSCX that are located on the surface of the protein, *e.g.*, hydrophilic regions. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, Proc. Nat. Acad. Sci. USA 78: 3824-3828; Kyte and Doolittle 1982, J. Mol. Biol. 157: 105-142, each incorporated herein by reference in their entirety.

TSCX polypeptides or derivatives, fragments, analogs or homologs thereof, may be utilized as immunogens in the generation of antibodies that immunospecifically-bind these protein components. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} and $F_{(ab')2}$ fragments, and an F_{ab} expression library. Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies to an TSCX protein sequence, or derivatives, fragments, analogs or homologs thereof. Some of these proteins are discussed below.

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by injection with the native protein, or a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, recombinantly expressed TSCX protein or a chemically synthesized TSCX polypeptide. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), human adjuvants such as *Bacille Calmette-Guerin* and *Corynebacterium parvum*, or similar immunostimulatory agents. If desired, the antibody molecules directed against TSCX can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as protein A chromatography to obtain the IgG fraction.

The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of TSCX. A monoclonal antibody composition thus typically displays a single binding affinity for a particular TSCX protein with which it immunoreacts. For preparation of monoclonal antibodies directed towards a particular TSCX protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture may be utilized. Such techniques include, but are not limited to, the hybridoma technique (see Kohler & Milstein, 1975 *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (see Kozbor, *et al.*, 1983 *Immunol Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, *et al.*, 1983. *Proc Natl Acad Sci USA* 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, *et al.*, 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to a TSCX protein (see *e.g.*, U.S. Patent No. 4,946,778). In addition, methods

can be adapted for the construction of F_{ab} expression libraries (see *e.g.*, Huse, *et al.*, 1989 *Science* 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a TSCX protein or derivatives, fragments, analogs or homologs thereof. Non-human antibodies can be "humanized" by techniques well known in the art. See *e.g.*, U.S. Patent No. 5,225,539. Antibody fragments that contain the idiotypes to a TSCX protein may be produced by techniques known in the art including, but not limited to: (*i*) an $F_{(ab')2}$ fragment produced by pepsin digestion of an antibody molecule; (*ii*) an F_{ab} fragment generated by reducing the disulfide bridges of an $F_{(ab')2}$ fragment; (*iii*) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (*iv*) F_v fragments.

Additionally, recombinant anti-TSCX antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT International Application No. PCT/US86/02269; European Patent Application No. 184,187; European Patent Application No. 171,496; European Patent Application No. 173,494; PCT International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; European Patent Application No. 125,023; Better *et al.*(1988) *Science* 240:1041-1043; Liu *et al.* (1987) *PNAS* 84:3439-3443; Liu *et al.* (1987) *J Immunol.* 139:3521-3526; Sun *et al.* (1987) *PNAS* 84:214-218; Nishimura *et al.* (1987) *Cancer Res* 47:999-1005; Wood *et al.* (1985) *Nature* 314:446-449; Shaw *et al.* (1988) *J Natl Cancer Inst.* 80:1553-1559); Morrison(1985) *Science* 229:1202-1207; Oi *et al.* (1986) *BioTechniques* 4:214; U.S. Pat. No. 5,225,539; Jones *et al.* (1986) *Nature* 321:552-525; Verhoeyan *et al.* (1988) *Science* 239:1534; and Beidler *et al.* (1988) *J Immunol* 141:4053-4060.

In one embodiment, methods for the screening of antibodies that possess the desired specificity include, but are not limited to, enzyme-linked immunosorbent assay (ELISA) and other immunologically-mediated techniques known within the art. In a specific embodiment, selection of antibodies that are specific to a particular domain of a TSCX protein is facilitated by generation of hybridomas that bind to the fragment of a TSCX protein possessing such a domain. Antibodies that are specific for one or more domains within a TSCX protein, *e.g.*, domains spanning the

above-identified conserved regions of TSCX family proteins, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

Anti-TSCX antibodies may be used in methods known within the art relating to the localization and/or quantitation of a TSCX protein (*e.g.,* for use in measuring levels of the TSCX protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like). In a given embodiment, antibodies for TSCX proteins, or derivatives, fragments, analogs or homologs thereof, that contain the antibody derived binding domain, are utilized as pharmacologically-active compounds [hereinafter "Therapeutics"].

An anti-TSCX antibody (*e.g.*, monoclonal antibody) can be used to isolate TSCX by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-TSCX antibody can facilitate the purification of natural TSCX from cells and of recombinantly produced TSCX expressed in host cells. Moreover, an anti-TSCX antibody can be used to detect TSCX protein (*e.g.*, in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the TSCX protein. Anti-TSCX antibodies can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

TSCX RECOMBINANT EXPRESSION VECTORS AND HOST CELLS

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding TSCX protein, or derivatives, fragments, analogs or homologs thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of

transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a linear or circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, that is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to includes promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired,

etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein (*e.g.*, TSCX proteins, mutant forms of TSCX, fusion proteins, etc.).

The recombinant expression vectors of the invention can be designed for expression of TSCX in prokaryotic or eukaryotic cells. For example, TSCX can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression of proteins in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, usually to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: (1) to increase expression of recombinant protein; (2) to increase the solubility of the recombinant protein; and (3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson (1988) *Gene* 67:31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant protein.

Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69:301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89).

One strategy to maximize recombinant protein expression in *E. coli* is to express the protein in a host bacteria with an impaired capacity to proteolytically cleave the recombinant

protein. See, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in *E. coli* (Wada *et al.*, (1992) *Nucleic Acids Res.* 20:211:1-7, 10-13, 19-34, 45-53, 58-85, 111-113, 120, 130, 132-134 and 13518). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

In another embodiment, the TSCX expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, *et al.*, (1987) *EMBO J* 6:229-234), pMFa (Kurjan and Herskowitz, (1982) *Cell* 30:933-943), pJRY88 (Schultz *et al.*, (1987) *Gene* 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (InVitrogen Corp, San Diego, Calif.).

Alternatively, TSCX can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith *et al.* (1983) *Mol Cell Biol* 3:2156-2165) and the pVL series (Lucklow and Summers (1989) *Virology* 170:31-39).

In yet another embodiment, a nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed (1987) *Nature* 329:840) and pMT2PC (Kaufman *et al.* (1987) *EMBO J* 6: 187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells. See, *e.g.*, Chapters 16 and 17 of Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (*e.g.*, tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin

promoter (liver-specific; Pinkert *et al.* (1987) *Genes Dev* 1:268-277), lymphoid-specific promoters (Calame and Eaton (1988) *Adv Immunol* 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore (1989) *EMBO J* 8:729-733) and immunoglobulins (Banerji *et al.* (1983) *Cell* 33:729-740; Queen and Baltimore (1983) *Cell* 33:741-748), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle (1989) *PNAS* 86:5473-5477), pancreas-specific promoters (Edlund *et al.* (1985) *Science* 230:912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss (1990) *Science* 249:374-379) and the α-fetoprotein promoter (Campes and Tilghman (1989) *Genes Dev* 3:537-546).

The invention further provides a recombinant expression vector comprising a DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to TSCX mRNA. Regulatory sequences operatively linked to a nucleic acid cloned in the antisense orientation can be chosen that direct the continuous expression of the antisense RNA molecule in a variety of cell types, for instance viral promoters and/or enhancers, or regulatory sequences can be chosen that direct constitutive, tissue specific or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid or attenuated virus in which antisense nucleic acids are produced under the control of a high efficiency regulatory region, the activity of which can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes see Weintraub *et al.*, "Antisense RNA as a molecular tool for genetic analysis," Reviews--Trends in Genetics, Vol. 1(1) 1986.

Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental

influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

A host cell can be any prokaryotic or eukaryotic cell. For example, TSCX protein can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells). Other suitable host cells are known to those skilled in the art.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding TSCX or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) an TSCX protein. Accordingly, the invention further provides methods for producing TSCX protein using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding TSCX has been introduced) in a suitable medium such that TSCX protein is

produced. In another embodiment, the method further comprises isolating TSCX from the medium or the host cell.

KITS AND NUCLEIC ACID COLLECTIONS FOR IDENTIFYING TSCX NUCLEIC ACIDS

In another aspect, the invention provides a kit useful for examining TSCXicity of agents. The kit can include nucleic acids that detect two or more TSCX sequences. In preferred embodiments, the kit includes reagents which detect 3, 4, 5, 6, 8, 10, 12, 15, 20, 25, 50, 100 or all of the TSCX nucleic acid sequences.

The invention also includes an isolated plurality of sequences which can identify one or more TSCX responsive nucleic acid sequences.

The kit or plurality may include, *e.g.*, sequence homologous to TSCX nucleic acid sequences, or sequences which can specifically identify one or more TSCX nucleic acid sequences.

EXAMPLES

EXAMPLE 1: EXPRESSION ANALYSIS OF ANTILEUKOPROTEASE IN VARIOUS TISSUES

The quantitative expression of NMB (GenBank Accession No: X04470; Table 1; TSC) was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR; TAQMAN®). RTQ PCR was performed on a Perkin-Elmer Biosystems ABI PRISM® 7700 Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing cells and cell lines from normal and cancer sources), and Panel 2 (containing samples derived from tissues, in particular from surgical samples, from normal and cancer sources).

First, the RNA samples were normalized to constitutively expressed genes such as β-actin and GAPDH. RNA (~50 ng total or ~1 ng polyA+) was converted to cDNA using the TAQMAN® Reverse Transcription Reagents Kit (PE Biosystems, Foster City, CA; Catalog No. N808-0234) and random hexamers according to the manufacturer's protocol. Reactions were performed in 20 ul and incubated for 30 min. at 48^{0}C. cDNA (5 ul) was then transferred to a separate plate for the TAQMAN® reaction using β-actin and GAPDH TAQMAN® Assay

Reagents (PE Biosystems; Catalog Nos. 4310881E and 4310884E, respectively) and TAQMAN® universal PCR Master Mix (PE Biosystems; Catalog No. 4304447) according to the manufacturer's protocol. Reactions were performed in 25 ul using the following parameters: 2 min. at 50°C; 10 min. at 95°C; 15 sec. at 95°C/1 min. at 60°C (40 cycles). Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100. The average CT values obtained for ß-actin and GAPDH were used to normalize RNA samples. The RNA sample generating the highest CT value required no further diluting, while all other samples were diluted relative to this sample according to their β-actin /GAPDH average CT values.

Normalized RNA (5 ul) was converted to cDNA and analyzed via TAQMAN® using One Step RT-PCR Master Mix Reagents (PE Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions. Probes and primers were designed for each assay according to Perkin Elmer Biosystem's *Primer Express* Software package (version I for Apple Computer's Macintosh Power PC) or a similar álgorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (T_m) range = 58°-60° C, primer optimal Tm = 59° C, maximum primer difference = 2° C, probe does not have 5' G, probe T_m must be 10° C greater than primer T_m, amplicon size 75 bp to 100 bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900 nM each, and probe, 200nM.

PCR conditions: Normalized RNA from each tissue and each cell line was spotted in each well of a 96 well PCR plate (Perkin Elmer Biosystems). PCR cocktails including two probes (a probe specific for the target clone and another gene-specific probe multiplexed with the target probe) were set up using 1X TaqMan™ PCR Master Mix for the PE Biosystems 7700, with 5 mM MgCl2, dNTPs (dA, G, C, U at 1:1:1:2 ratios), 0.25 U/ml AmpliTaq Gold™ (PE Biosystems), and 0.4 U/µl RNase inhibitor, and 0.25 U/µl reverse transcriptase. Reverse transcription was performed at 48° C for 30 minutes followed by amplification/PCR cycles as follows: 95° C 10

min, then 40 cycles of 95° C for 15 seconds, 60° C for 1 minute.

In the results for Panel 1, the following abbreviations are used:

ca. = carcinoma,

* = established from metastasis,

met = metastasis,

s cell var= small cell variant,

non-s = non-sm =non-small,

squam = squamous,

pl. eff = pl effusion = pleural effusion,

glio = glioma,

astro = astrocytoma, and

neuro = neuroblastoma.

Panel 2

The plates for Panel 2 generally include 2 control wells and 94 test samples composed of RNA or cDNA isolated from human tissue procured by surgeons working in close cooperation with the National Cancer Institute's Cooperative Human Tissue Network (CHTN) or the National Disease Research Initiative (NDRI). The tissues are derived from human malignancies and in cases where indicated many malignant tissues have "matched margins" obtained from noncancerous tissue just adjacent to the tumor. These are termed normal adjacent tissues and are denoted "NAT" in the results below. The tumor tissue and the "matched margins" are evaluated by two independent pathologists (the surgical pathologists and again by a pathologists at NDRI or CHTN). This analysis provides a gross histopathological assessment of tumor differentiation grade. Moreover, most samples include the original surgical pathology report that provides information regarding the clinical stage of the patient. These matched margins are taken from the tissue surrounding (i.e. immediately proximal) to the zone of surgery (designated "NAT", for normal adjacent tissue, in Table 4). In addition, RNA and cDNA samples were obtained from various human tissues derived from autopsies performed on elderly people or sudden death victims (accidents, etc.). These tissue were ascertained to be free of disease and were purchased from various commercial sources such as Clontech (Palo Alto, CA), Research Genetics, and Invitrogen.

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs that would be indicative of degradation products. Samples are controlled against genomic DNA contamination by RTQ PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

The TaqMan™ expression profiles of NMB were generated using a specific gene probes and primer set (Ag 817)as shown below:

Ag 817 forward : 5'-TCAATGGAACCTTCAGCCTTA-3'

ProbeTET :5'-CTCACTGTGAAAGCTGCAGCACCAG-3'-TAMRA

Reverse : 5'-GAAGGGGTGGGTTTTGAAG-3'

The results shown in Table 2 (see below) relate to 41 normal human tissues and 55 human cancer cell lines and demonstrate the high expression of NMB in melanomas cell lines and overexpression in the breast cancer cell line MDA-N. The results shown in Table 3 (see below) relate to additional tumor tissues, many of which are matched with normal adjacent tissue (NAT), as defined by the operating surgeon that obtained the samples. It reveals that NMB is overexpressed in 9/9 kidney tumors compared either with normal kidney or NAT. This analysis corroborates the GeneCalling™ results which originally identified the expression of NMB that NMB is also overexpressed in some of the lung carcinoma tissues compared with NATs and 2 melanoma metastasis compared with NAT.

NCI's CGAP Sage analysis indicates that NMB is expressed in several glioblastoma (H392, pooled GBM, GBMH1110), and in 1 malignant breast tumor (SKBR3), in accordance with panel 1 TaqMan analysis. NCI data for EST expression, called "body map" , reveals that NMB is expressed in Schwann cells, in adenocarcinoma and s.cell carcinoma.

Based on NMB's gene expression profile and its homology with pMEL17, it is anticipated that for a subset of human tumors including renal cell carcinomas, lung carcinomas, melanomas and CNS cancers, successful targeting of NMB using a monoclonal antibody will have an inhibitory effect on tumor growth, matrix invasion and metastatic dissemination. Furthermore, targeting of NMB will have a therapeutic effect on the TSC disease.

Furthermore, in consideration of NMB potential enzymatic activity, NMB could be used as

a target for screening a small molecule drug.

In summary, these results demonstate the relevance of NMB as a therapeutic target for the treatment of TSC is strenghtened by its expression/overexpression in several tissues that are affected in TSC

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EXAMPLE 2: THERAPEUTIC TARGETING OF CYR61

Based on CYR61's gene expression profile, it is anticipated that for a subset of human tumors including renal cell carcinomas, lung carcinomas, melanomas and CNS cancers, successful
10 targeting of CYR61 using a monoclonal antibody will have an inhibitory effect on tumor growth, matrix invasion and metastatic dissemination. Furthermore, targeting of CYR61 will have a therapeutic effect on the TSC disease.

EXAMPLE 3: THERAPEUTIC TARGETING OF NET-7

NET-7 is overexpressed by a breast cancer cell lines and it is regulated by estradiol
15 treatment of a ER positive cell line MCF7. Based on NET-7's gene expression profile, it is anticipated that for a subset of human tumors specifically breast tumors, successful targeting of NET-7 using a monoclonal antibody will have an inhibitory effect on tumor growth, matrix invasion and metastatic dissemination. Furthermore, targeting of NET-7 will have a therapeutic effect on the TSC disease adrenomedullin precursor (and Receptor activity modifying protein 1)

20 NET-7 has potent and long-lasting vasodilatory effects in several vascular systems. In addition to adrenomedullin, another hypotensive peptide, proadrenomedullin-derived peptide (PAMP), was also found to be processed from the adrenomedullin precursor. PAMP inhibits neural transmission at peripheral sympathetic nerve endings, although adrenomedullin directly dilates vascular smooth muscle. Adrenomedullin might participate in the pathogenesis of
25 hypertension, renal failure and congestive heart failure. Receptor activity-modifying proteins (RAMPs) are single-transmembrane proteins that transport the calcitonin receptor-like receptor (CRLR) to the cell surface. RAMP 1-transported CRLR is a calcitonin gene-related peptide (CGRP) receptor. RAMP1 is downregulated in NSC.Because of its activities, overexpression of adrenomedullin precursor by TSC patients might explain some of the TSC

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

Table 2 : Taq Man results for PANEL 1	
Tissue Name	Rel. Expr., % 1.2tm958t_ag817
Endothelial cells	0
Heart (fetal)	5.4
Pancreas	6
Pancreatic ca. CAPAN 2	0
Adrenal Gland (new lot*)	2.7
Thyroid	19.3
Salavary gland	2.7
Pituitary gland	3.7
Brain (fetal)	0.8
Brain (whole)	2.4
Brain (amygdala)	1.6
Brain (cerebellum)	0.4
Brain (hippocampus)	1.3
Brain (thalamus)	1.1
Cerebral Cortex	1.2
Spinal cord	7.6
CNS ca. (glio/astro) U87-MG	27.2
CNS ca. (glio/astro) U-118-MG	13.5
CNS ca. (astro) SW1783	0.4
CNS ca.* (neuro; met) SK-N-AS	0.7
CNS ca. (astro) SF-539	52.9
CNS ca. (astro) SNB-75	7
CNS ca. (glio) SNB-19	1.3
CNS ca. (glio) U251	4.9
CNS ca. (glio) SF-295	11
Heart	17.1
Skeletal Muscle (new lot*)	5.7
Bone marrow	0.8
Thymus	9.9
Spleen	5
Lymph node	25.7
Colorectal	8.2
Stomach	5.6
Small intestine	8.1
Colon ca. SW480	0
Colon ca.* (SW480 met)SW620	0
Colon ca. HT29	0
Colon ca. HCT-116	0
Colon ca. CaCo-2	0
83219 CC Well to Mod Diff (ODO3866)	2.4
Colon ca. HCC-2998	0.1
Gastric ca.* (liver met) NCI-N87	18.2
Bladder	8.1
Trachea	7.4

Kidney		3.1
Kidney (fetal)		1.7
Renal ca.	786-0	0
Renal ca.	A498	4.7
Renal ca.	RXF 393	1.5
Renal ca.	ACHN	0
Renal ca.	UO-31	1.8
Renal ca.	TK-10	0
Liver		2.5
Liver (fetal)		2.3
Liver ca. (hepatoblast)	HepG2	0
Lung		21

Table 3 Taq Man Results for Panel 2	
Tissue Name	Rel. Expr., % 2tm1063t_ag817
Normal Colon GENPAK 061003	11.8
83219 CC Well to Mod Diff (ODO3866)	0
83220 CC NAT (ODO3866)	9.1
83221 CC Gr.2 rectosigmoid (ODO3868)	1.4
83222 CC NAT (ODO3868)	7.1
83235 CC Mod Diff (ODO3920)	1.2
83236 CC NAT (ODO3920)	1.2
83237 CC Gr.2 ascend colon (ODO3921)	4.8
83238 CC NAT (ODO3921)	5.8
83241 CC from Partial Hepatectomy (ODO4309)	7.8
83242 Liver NAT (ODO4309)	2.9
87472 Colon mets to lung (OD04451-01)	14.6
87473 Lung NAT (OD04451-02)	19.8
Normal Prostate Clontech A+ 6546-1	8.8
84140 Prostate Cancer (OD04410)	2.9
84141 Prostate NAT (OD04410)	0.7
87073 Prostate Cancer (OD04720-01)	1
87074 Prostate NAT (OD04720-02)	1.5
Normal Lung GENPAK 061010	49.3
83239 Lung Met to Muscle (ODO4286)	74.7
83240 Muscle NAT (ODO4286)	6.5
84136 Lung Malignant Cancer (OD03126)	10.4
84137 Lung NAT (OD03126)	4.6
84871 Lung Cancer (OD04404)	27.7
84872 Lung NAT (OD04404)	7.9
84875 Lung Cancer (OD04565)	41.8
84876 Lung NAT (OD04565)**	3.8
85950 Lung Cancer (OD04237-01)	10.1
85970 Lung NAT (OD04237-02)	1.5
83255 Ocular Mel Met to Liver (ODO4310)	77.4
83256 Liver NAT (ODO4310)	1.8
84139 Melanoma Mets to Lung (OD04321)	53.6
84138 Lung NAT (OD04321)	5.8
Normal Kidney GENPAK 061008	10.1
83786 Kidney Ca, Nuclear grade 2 (OD04338)	22.5
83787 Kidney NAT (OD04338)	1.3
83788 Kidney Ca Nuclear grade 1/2 (OD04339)	17.2
83789 Kidney NAT (OD04339)	2
83790 Kidney Ca, Clear cell type (OD04340)	11.3
83791 Kidney NAT (OD04340)	3.7
83792 Kidney Ca, Nuclear grade 3 (OD04348)	12.1
83793 Kidney NAT (OD04348)	1.9
87474 Kidney Cancer (OD04622-01)	19.6
87475 Kidney NAT (OD04622-03)	9
85973 Kidney Cancer (OD04450-01)	54.7

85974 Kidney NAT (OD04450-03)	2.7
Kidney Cancer Clontech 8120613	67.8
Kidney NAT Clontech 8120614	5.8
Kidney Cancer Clontech 9010320	56.3
Kidney NAT Clontech 9010321	7.2
Kidney Cancer Clontech 8120607	100
Kidney NAT Clontech 8120608	10.2
Normal Uterus GENPAK 061018	11.5
Uterus Cancer GENPAK 064011	2
Normal Thyroid Clontech A+ 6570-1**	44.4
Thyroid Cancer GENPAK 064010	90.1
Thyroid Cancer INVITROGEN A302152	10.9
Thyroid NAT INVITROGEN A302153	8.3
Normal Breast GENPAK 061019	2.4
84877 Breast Cancer (OD04566)	5.5
Breast Cancer Res. Gen. 1024	7.1
85975 Breast Cancer (OD04590-01)	1.7
85976 Breast Cancer Mets (OD04590-03)	2
87070 Breast Cancer Metastasis (OD04655-05)	1.6
GENPAK Breast Cancer 064006	3.4
Breast Cancer Clontech 9100266	11.1
Breast NAT Clontech 9100265	7.7
Breast Cancer INVITROGEN A209073	11
Breast NAT INVITROGEN A2090734	3.2
Normal Liver GENPAK 061009	6
Liver Cancer Research Genetics RNA 1026	36.3
Liver Cancer Research Genetics RNA 1025	4
Paired Liver Cancer Tissue Research Genetics RNA 6004-T	10.4
Paired Liver Tissue Research Genetics RNA 6004-N	32.1
Paired Liver Cancer Tissue Research Genetics RNA 6005-T	44.4
Paired Liver Tissue Research Genetics RNA 6005-N	40.6
Liver Cancer GENPAK 064003	18.4
Normal Bladder GENPAK 061001	19.9
Bladder Cancer Research Genetics RNA 1023	17
87071 Bladder Cancer (OD04718-01)	1.4
87072 Bladder Normal Adjacent (OD04718-03)	0.9
Bladder Cancer INVITROGEN A302173	43.8
Normal Ovary Res. Gen.	39.5
Ovarian Cancer GENPAK 064008	10.8
87492 Ovary Cancer (OD04768-07)	5
87493 Ovary NAT (OD04768-08)	6.2
Normal Stomach GENPAK 061017	37.4
Gastric Cancer Clontech 9060358**	7.4
NAT Stomach Clontech 9060359	14.6
Gastric Cancer Clontech 9060397	40.9
NAT Stomach Clontech 9060396	9.9
Gastric Cancer Clontech 9060395	20.9
NAT Stomach Clontech 9060394	22.2

Gastric Cancer GENPAK 064005	8.6
genomic DNA control	4.5
Chemistry Control	0.1